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 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3  SEP 09   CA/CAplus records now contain indexing from 1907 to the
                   present
 NEWS  4  DEC 08   INPADOC: Legal Status data reloaded
 NEWS  5  SEP 29   DISSABS now available on STN
 NEWS  6  OCT 10   PCTFULL: Two new display fields added
 NEWS  7  OCT 21   BIOSIS file reloaded and enhanced
 NEWS  8  OCT 28   BIOSIS file segment of TOXCENTER reloaded and enhanced
 NEWS  9  NOV 24   MSDS-CCOHS file reloaded
 NEWS 10  DEC 08   CABA reloaded with left truncation
 NEWS 11  DEC 08   IMS file names changed
 NEWS 12  DEC 09   Experimental property data collected by CAS now available
                   in REGISTRY
 NEWS 13  DEC 09   STN Entry Date available for display in REGISTRY and CA/CAplus
 NEWS 14  DEC 17   DGENE: Two new display fields added
 NEWS 15  DEC 18   BIOTECHNO no longer updated
 NEWS 16  DEC 19   CROPU no longer updated; subscriber discount no longer
                   available
 NEWS 17  DEC 22   Additional INPI reactions and pre-1907 documents added to CAS
                   databases
 NEWS 18  DEC 22   IFIPAT/IFIUDB/IFICDB reloaded with new data and search fields
 NEWS 19  DEC 22   ABI-INFORM now available on STN
 NEWS 20  JAN 27   Source of Registration (SR) information in REGISTRY updated
                   and searchable
 NEWS 21  JAN 27   A new search aid, the Company Name Thesaurus, available in
                   CA/CAplus
 NEWS 22  FEB 05   German (DE) application and patent publication number format
                   changes
 NEWS 23  MAR 03   MEDLINE and LMEDLINE reloaded
 NEWS 24  MAR 03   MEDLINE file segment of TOXCENTER reloaded
 NEWS 25  MAR 03   FRANCEPAT now available on STN
 NEWS 26  MAR 29   Pharmaceutical Substances (PS) now available on STN
 NEWS 27  MAR 29   WPIFV now available on STN
 NEWS 28  MAR 29   No connect hour charges in WPIFV until May 1, 2004
 NEWS 29  MAR 29   New monthly current-awareness alert (SDI) frequency in RAPRA

 NEWS EXPRESS  MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 3 MARCH 2004
 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS INTER    General Internet Information
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FILE 'HOME' ENTERED AT 16:18:42 ON 31 MAR 2004

=> file medline, uspatful, dgene, embase, wpids, fsta, jicst, biosis
FILE 'DGENE' TEMPORARILY UNAVAILABLE
If this message appears repeatedly, please notify the Help Desk.
Enter "HELP STN" for information on contacting the nearest STN Help Desk by telephone or via SEND in the STNMAIL file.
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FILE 'DGENE' TEMPORARILY UNAVAILABLE
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FILE 'WPIDS' TEMPORARILY UNAVAILABLE
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FILE 'FSTA' TEMPORARILY UNAVAILABLE
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=> file medline, biosis, embase, wpids, fsta, hcaplus, jicst
FILE 'WPIDS' TEMPORARILY UNAVAILABLE
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ENTER A FILE NAME OR (IGNORE):jicst
FILE 'FSTA' TEMPORARILY UNAVAILABLE
If this message appears repeatedly, please notify the Help Desk.
Enter "HELP STN" for information on contacting the nearest STN Help Desk by telephone or via SEND in the STNMAIL file.
ENTER A FILE NAME OR (IGNORE):end

=> file medline, biosis, wpids, fsta, uspatful, embase, hcaplus
FILE 'WPIDS' TEMPORARILY UNAVAILABLE
If this message appears repeatedly, please notify the Help Desk.
Enter "HELP STN" for information on contacting the nearest STN Help Desk by telephone or via SEND in the STNMAIL file.
ENTER A FILE NAME OR (IGNORE):log y
'LOG' IS NOT A VALID FILE NAME
Enter "HELP FILE NAMES" at an arrow prompt (=>) for a list of files that are available. If you have requested multiple files, you can specify a corrected file name or you can enter "IGNORE" to continue accessing the remaining file names entered.
ENTER A FILE NAME OR (IGNORE):end

=> file biosis
COST IN U.S. DOLLARS SINCE FILE TOTAL

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                                                        ENTRY     SESSION
FULL ESTIMATED COST                                      1.05        1.05

FILE 'BIOSIS' ENTERED AT 16:21:50 ON 31 MAR 2004
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FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 24 March 2004 (20040324/ED)

FILE RELOADED: 19 October 2003.

=> s nonarticular cartilage adj repair or regeneration
           112 NONARTICULAR
         29840 CARTILAGE
           305 ADJ
         80952 REPAIR
             0 NONARTICULAR CARTILAGE ADJ REPAIR
               (NONARTICULAR(W)CARTILAGE(W)ADJ(W)REPAIR)
         65221 REGENERATION
L1       65221 NONARTICULAR CARTILAGE ADJ REPAIR OR REGENERATION

=> s l1 and defect locu
         82644 DEFECT
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             0 DEFECT LOCU
               (DEFECT(W)LOCU)
L2           0 L1 AND DEFECT LOCU

=> s l1 and defect locus
         82644 DEFECT
         91526 LOCUS
             4 DEFECT LOCUS
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L3           0 L1 AND DEFECT LOCUS

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         91526 LOCUS
L4           4 DEFECT LOCUS
               (DEFECT(W)LOCUS)

=> s l4 and repair
         80952 REPAIR
L5           0 L4 AND REPAIR

=> s l4 and cartilage repair
         29840 CARTILAGE
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           329 CARTILAGE REPAIR
               (CARTILAGE(W)REPAIR)
L6           0 L4 AND CARTILAGE REPAIR

=> d his

     (FILE 'HOME' ENTERED AT 16:18:42 ON 31 MAR 2004)

     FILE 'BIOSIS' ENTERED AT 16:21:50 ON 31 MAR 2004
L1        65221 S NONARTICULAR CARTILAGE ADJ REPAIR OR REGENERATION
L2            0 S L1 AND DEFECT LOCU
L3            0 S L1 AND DEFECT LOCUS
L4            4 S DEFECT LOCUS
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L5              0 S L4 AND REPAIR
L6              0 S L4 AND CARTILAGE REPAIR

=> s l1 and BMP
          3953 BMP
L7         144 L1 AND BMP

=> s l7 and OP
          4683 OP
L8           15 L7 AND OP

=> d l8 ti abs ibib tot

L8   ANSWER 1 OF 15  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   EFFECTS OF BMP-7 ON LIVER REGENERATION AFTER PARTIAL
     HEPATECTOMY IN MICE.
AB   Background: Acute liver failure is characterized by loss of liver function
     and recovery is only possible through adequate liver regeneration
     . TGF-beta1, a known inhibitor of proliferation for many kinds of cells,
     is also the most powerful inhibitor of hepatocyte proliferation in vitro
     and in vivo. Bone morphogenic protein-7 (BMP-7), also referred
     to as Osteogenic protein-1 (OP-1), is a member of the TGF-beta1
     superfamily. We have previously demonstrated that BMP-7
     functions as an antagonist of TGF-beta1 in murine models of chronic renal
     failure, inhibits TGF-beta1 induced pro-fibrotic responses. Therefore, in
     the present study we tested the capacity of BMP-7 to accelerate
     liver regeneration. Methods: 70% partial hepatectomy (PH) was
     performed by removal of the left and middle lobes of livers in 61 CD-1
     mice. Three groups were studied; 300 mg/kg of BMP-7
     intraperitoneally (IP) every other day; a placebo IP injection and a sham
     surgery group. Mice were sacrificed after 2, 4, 7 and 10 days after
     surgery and regeneration potential was assessed by
     regeneration rate, tissue morphology, BrdU incorporation-
     proliferation index, and apoptosis using TUNEL assay. In addition
     TGF-beta1 and hepatic growth factor (HGF) were also assessed in the tissue
     samples by Western blot and immunohistochemistry. Liver function was
     analyzed by serum ALT, AST and total bilirubin measurement. Results:
     Systemic administration of BMP-7 lead to a dramatic acceleration
     of liver regeneration at the groups of 2 days, 4 days and 7 days
     after PH, according to the BrdU index and liver regeneration
     rate, compared with control group and sham group. Hepatocytes, which can
     express ALK-3 (type I receptor of BMP-7), become the main source
     of TGF-beta1 after PH. The expression HGF was not affected by the
     administration of BMP-7, however, the increase of TGF-beta1
     expression after PH was down-regulated. Apoptosis of hepatocytes also
     decreased at 7 days and 10days after PH in the BMP-7 treated
     groups. Liver function was improved at 4 days and 7 days after PH in the
     treated group. Conclusion: BMP-7 is an effective non-toxic
     protein capable of significant acceleration of liver regeneration
     . BMP-7 appears to promote regeneration by down
     regulation of TGF-beta1 and by reducing hepatocyte apoptosis. Further
     studies are needed to evaluate a potential clinical role for BMP
     -7 in acute liver failure..
ACCESSION NUMBER:   2004:25651  BIOSIS
DOCUMENT NUMBER:    PREV200400024050
TITLE:              EFFECTS OF BMP-7 ON LIVER REGENERATION
                    AFTER PARTIAL HEPATECTOMY IN MICE.
AUTHOR(S):          Yang, Changqing [Reprint Author]; Sugimoto, Hikaru [Reprint
                    Author]; Akulapalli, Sudhakar [Reprint Author]; Giraldo,
                    Mauricio [Reprint Author]; Afdhal, Nezam [Reprint Author];
                    Zeisberg, Michael [Reprint Author]; Kalluri, Raghu [Reprint
                    Author]
CORPORATE SOURCE:   Boston, MA, USA
SOURCE:             Digestive Disease Week Abstracts and Itinerary Planner,
```

(2003) Vol. 2003, pp. Abstract No. M1440. e-file.
Meeting Info.: Digestive Disease 2003. FL, Orlando, USA. May 17-22, 2003. American Association for the Study of Liver Diseases; American Gastroenterological Association; American Society for Gastrointestinal Endoscopy; Society for Surgery of the Alimentary Tract.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 31 Dec 2003
Last Updated on STN: 31 Dec 2003

L8 ANSWER 2 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Neurotrophic properties of olfactory ensheathing glia.
AB Olfactory ensheathing cells (OEC) constitute a specialized population of glia that accompany primary olfactory axons and have been reported to facilitate axonal **regeneration** after spinal cord injury in vivo. In the present report we describe OEC neurotrophic factor expression and neurotrophic properties of OECs in vitro. Investigation of the rat olfactory system during development and adulthood by radioactive in situ hybridization revealed positive labeling in the olfactory nerve layer for the neurotrophic molecules S-100beta, CNTF, **BMP**-7/**OP**-1, and artemin, as well as for the neurotrophic factor receptors RET and TrkC. Ribonuclease protection assay of cultured OEC revealed expression of NGF, BDNF, GDNF, and CNTF mRNA, while NT3 and NT4 mRNA were not detectable. In vitro bioassays of neurotrophic activity involved coculturing of adult OEC with embryonic chick ganglia and demonstrated increased neurite outgrowth from sympathetic, ciliary, and Remak's ganglia. However, when culturing the ganglia with OEC-conditioned medium, neurite outgrowth was not stimulated to any detectable extent. Our results suggest that the neurotrophic properties of OEC may involve secretion of neurotrophic molecules but that cellular interactions are crucial.
ACCESSION NUMBER: 2003:262693 BIOSIS
DOCUMENT NUMBER: PREV200300262693
TITLE: Neurotrophic properties of olfactory ensheathing glia.
AUTHOR(S): Lipson, Adam C.; Widenfalk, Johan; Lindqvist, Eva; Ebendal, Ted; Olson, Lars [Reprint Author]
CORPORATE SOURCE: Department of Neuroscience, Karolinska Institutet, S-171 77, Stockholm, Sweden
Lars.Olson@neuro.ki.se
SOURCE: Experimental Neurology, (April 2003) Vol. 180, No. 2, pp. 167-171. print.
CODEN: EXNEAC. ISSN: 0014-4886.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Jun 2003
Last Updated on STN: 4 Jun 2003

L8 ANSWER 3 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Bone morphogenic protein-7, a member of the TGF-beta superfamily, accelerates liver **regeneration** after partial hepatectomy in mice.
ACCESSION NUMBER: 2002:618793 BIOSIS
DOCUMENT NUMBER: PREV200200618793
TITLE: Bone morphogenic protein-7, a member of the TGF-beta superfamily, accelerates liver **regeneration** after partial hepatectomy in mice.
AUTHOR(S): Yang, Changqing [Reprint author]; Sugimoto, Hikaru [Reprint author]; Giraldo, Mauricio [Reprint author]; Afdhal, Nezam [Reprint author]; Zeisberg, Michael [Reprint author]; Kalluri, Raghu [Reprint author]
CORPORATE SOURCE: Beth Israel Deaconess Medical Center and Harvard Medical

School, Boston, MA, USA
SOURCE: Hepatology, (October, 2002) Vol. 36, No. 4 Part 2, pp. 470A. print.
Meeting Info.: 53rd Annual Meeting on the Liver. BOSTON, MA, USA. November 01-05, 2002.
CODEN: HPTLD9. ISSN: 0270-9139.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Dec 2002
Last Updated on STN: 4 Dec 2002

L8 ANSWER 4 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Periodontal tissue **regeneration** by combined applications of recombinant human osteogenic protein-1 and bone morphogenetic protein-2. A pilot study in Chacma baboons (Papio ursinus).
AB Native and recombinant human bone morphogenetic/osteogenic proteins (BMPs/OPs) singly initiate bone induction in vivo. The finding of synchronous but spatially different BMPs/OPs expression during periodontal tissue morphogenesis suggests novel therapeutic approaches using morphogen combinations based on recapitulation of embryonic development. Twelve furcation defects prepared in the first and second mandibular molars of three adult baboons (Papio ursinus) were used to assess whether qualitative histological aspects of periodontal tissue **regeneration** could be enhanced and tissue morphogenesis modified by combined or single applications of recombinant hOP-1 and hBMP-2. Doses of BMPs/OPs were 100 mug of each protein per 1 g of insoluble collagenous bone matrix as carrier. Approximately 200 mg of carrier matrix was used per furcation defect. Undercalcified sections cut for histological analysis 60 d after healing of hOP-1-treated specimens showed substantial cementogenesis with scattered remnants of the collagenous carrier. hBMP-2 applied alone induced greater amounts of mineralized bone and osteoid when compared to hOP-1 alone or to combined morphogen applications. Combined applications of hOP-1 and hBMP-2 did not enhance alveolar bone **regeneration** or new attachment formation over and above the single applications of the morphogens. The results of this study, which is the first to attempt to address the structure-activity relationship amongst **BMP/OP** family members, indicate that tissue morphogenesis induced by hOP-1 and hBMP-2 is qualitatively different when the morphogens are applied singly, with hOP-1 inducing substantial cementogenesis. hBMP-2 treated defects, on the other hand, showed limited cementum formation but a temporal enhancement of alveolar bone **regeneration** and remodelling. The demonstration of therapeutic mosaicism in periodontal **regeneration** will require extensive testing of ratios and doses of recombinant morphogen combinations for optimal tissue engineering in clinical contexts.
ACCESSION NUMBER: 2001:444101 BIOSIS
DOCUMENT NUMBER: PREV200100444101
TITLE: Periodontal tissue **regeneration** by combined applications of recombinant human osteogenic protein-1 and bone morphogenetic protein-2. A pilot study in Chacma baboons (Papio ursinus).
AUTHOR(S): Ripamonti, Ugo [Reprint author]; Crooks, Jean; Petit, Jean-Claude; Rueger, David C.
CORPORATE SOURCE: Bone Research Unit, MRC/University of the Witwatersrand, Medical School, 7 York Road, Parktown, 2193, Johannesburg, South Africa
177ripa@chiron.wits.ac.za
SOURCE: European Journal of Oral Sciences, (August, 2001) Vol. 109, No. 4, pp. 241-248. print.
ISSN: 0909-8836.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 19 Sep 2001

Last Updated on STN: 22 Feb 2002

L8 ANSWER 5 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Growth factor delivery for tissue engineering.
AB A tissue-engineered implant is a biologic-biomaterial combination in which some component of tissue has been combined with a biomaterial to create a device for the restoration or modification of tissue or organ function. Specific growth factors, released from a delivery device or from co-transplanted cells, would aid in the induction of host paraenchymal cell infiltration and improve engraftment of co-delivered cells for more efficient tissue **regeneration** or ameliorate disease states. The characteristic properties of growth factors are described to provide a biological basis for their use in tissue engineered devices. The principles of polymeric device development for therapeutic growth factor delivery in the context of tissue engineering are outlined. A review of experimental evidence illustrates examples of growth factor delivery from devices such as micropaticles, scaffolds, and encapsulated cells, for their use in the application areas of musculoskeletal tissue, neural tissue, and hepatic tissue.
ACCESSION NUMBER: 2000:385919 BIOSIS
DOCUMENT NUMBER: PREV200000385919
TITLE: Growth factor delivery for tissue engineering.
AUTHOR(S): Babensee, Julia E.; McIntire, Larry V.; Mikos, Antonios G. [Reprint author]
CORPORATE SOURCE: Department of Bioengineering, Institute of Biosciences and Bioengineering, Rice University, Houston, TX, 77005, USA
SOURCE: Pharmaceutical Research (New York), (May, 2000) Vol. 17, No. 5, pp. 497-504. print.
CODEN: PHREEB. ISSN: 0724-8741.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Sep 2000
Last Updated on STN: 8 Jan 2002

L8 ANSWER 6 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Bone morphogenetic proteins in human bone **regeneration**.
AB Recently, the first clinical reports on bone **regeneration** by two recombinant human bone morphogenetic proteins (rhBMPs), **BMP**-2 and **BMP**-7 (also named osteogenic protein-1, **OP**-1) have been published (1-4). Although both BMPs were able to support bone **regeneration**, a significant variation in individual response was observed with both proteins. Animal studies and laboratory experiments reveal a number of conditions that influence the osteoinductivity of **BMP**, such as **BMP** concentration, carrier properties and influence of local and systemic growth factors and hormones. In this paper, these studies and the clinical reports are reviewed, and the conditions that modulate the **BMP**-dependent osteoinduction are discussed. The information may provide clues as to how the performance of recombinant human **BMP** as bone-graft substitute in humans can be improved.
ACCESSION NUMBER: 2000:126320 BIOSIS
DOCUMENT NUMBER: PREV200000126320
TITLE: Bone morphogenetic proteins in human bone **regeneration**.
AUTHOR(S): Groeneveld, E. H. J.; Burger, E. H. [Reprint author]
CORPORATE SOURCE: Department of Oral Cell Biology, ACTA-Vrije Universiteit, Van der Boechorststraat 7, 1081 BT, Amsterdam, Netherlands
SOURCE: European Journal of Endocrinology, (Jan., 2000) Vol. 142, No. 1, pp. 9-21. print.
ISSN: 0804-4643.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English

ENTRY DATE: Entered STN: 5 Apr 2000
Last Updated on STN: 4 Jan 2002

L8 ANSWER 7 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Use of recombinant human osteogenic protein-1 for the repair of subchondral defects in articular cartilage in goats.
AB The objective of this pilot study was to examine in vivo the potential of recombinant human osteogenic protein-1 (rhOP-1, also called bone morphogenetic protein-7, **BMP**-7) for treatment of subchondral lesions by induction of new hyaline cartilage formation. Subchondral left knee defects in 17 mature goats were treated with fresh coagulated blood mixed with (1) rhOP-1 combined with collagen (**OP**-1 device, 400 mug/mL); (2) rhOP-1 alone (**OP**-1 peptide, 200 mug/mL); (3) **OP**-1 device with small particles of autologous ear perichondrium; (4) **OP**-1 peptide with small particles of autologous ear perichondrium; or (5) autologous ear perichondrium alone (controls). rhOP-1 was combined with either collagen (**OP**-1 device) or not (**OP**-1 peptide). The defects were closed with a periosteal flap. The formation of cartilage tissue was studied by histologic and biochemical evaluation at 1, 2, and 4 months after implantation. One and 2 months after implantation there were no obvious differences between control and rhOP-1-treated defects. Four months after implantation, only one out of three controls (without rhOP-1) showed beginning signs of cartilage formation while all four rhOP-1-treated defects were completely or partly filled with cartilage. A significant linear relationship was found between rhOP-1 concentration and the total amount of aggrecan in the defects. These results suggest that implantation of rhOP-1 promotes cartilage formation in subchondral defects in goats at 4 months after implantation. Therefore, rhOP-1 could be a novel factor for **regeneration** of cartilage in articular cartilage defects.
ACCESSION NUMBER: 2000:126295 BIOSIS
DOCUMENT NUMBER: PREV200000126295
TITLE: Use of recombinant human osteogenic protein-1 for the repair of subchondral defects in articular cartilage in goats.
AUTHOR(S): Louwerse, R. T.; Heyligers, I. C.; Klein-Nulend, J.; Sugihara, S.; van Kampen, G. P. J.; Semeins, C. M.; Goei, S. W.; de Koning, M. H. M. T.; Wuisman, P. I. J. M.; Burger, E. H. [Reprint author]
CORPORATE SOURCE: Department of Oral Cell Biology, ACTA-Vrije Universiteit, Van der Boechorststraat 7, 1081 BT, Amsterdam, Netherlands
SOURCE: Journal of Biomedical Materials Research, (March 15, 2000) Vol. 49, No. 4, pp. 506-516. print.
CODEN: JBMRBG. ISSN: 0021-9304.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Apr 2000
Last Updated on STN: 4 Jan 2002

L8 ANSWER 8 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Immunolocalization of Bone Morphogenetic Protein-2 and -3 and Osteogenic Protein-1 during murine tooth root morphogenesis and in other craniofacial structures.
AB The distribution of Bone Morphogenetic Protein-2, and -3 (**BMP**-2 and **BMP**-3) and Osteogenic Protein-1 (**OP**-1, also known as **BMP**-7) during root morphogenesis and in other craniofacial structures was examined in sections of 12- to 18-d-old mouse heads using polyclonal and monoclonal antibodies. **BMP**-3 and **OP**-1 were localized in alveolar bone, cementum, and periodontal ligament, whereas **BMP**-2 was only localized in the alveolar bone of periodontium. All three BMPs were localized in predentine, dentine, odontoblasts, osteoblasts, osteocytes, osteoid, cartilage, chondrocytes and spiral limbus. **BMP**-2 and **OP**-1 were also localized in spiral ligament and interdentate cells of the cochlea, whilst

BMP-3 was restricted to the spiral ganglion. **BMP**-3 was also localized in ducts of submandibular and sublingual salivary glands, acini of the lacrimal gland, Purkinje cells in the cerebellum, nerve fibres of the cerebellum and brain, afferent cells of the dorsal root ganglia, inferior alveolar nerve, and peripheral processes of the vestibulocochlear nerve. **OP**-1 was also localized in hair and whisker follicles, sclera of the eye and in ameloblasts. The demonstration of **BMP**-3 in the nervous system suggests that this protein may be neurotrophic during development and maintenance of the nervous system. The composite expression of BMPs/OPs during periodontal tissue morphogenesis suggests that optimal therapeutic **regeneration** may entail the combined use of different BMPs/OPs.

ACCESSION NUMBER: 1999:513642 BIOSIS
DOCUMENT NUMBER: PREV199900513642
TITLE: Immunolocalization of Bone Morphogenetic Protein-2 and -3 and Osteogenic Protein-1 during murine tooth root morphogenesis and in other craniofacial structures.
AUTHOR(S): Thomadakis, Georgios; Ramoshebi, Lentsha Nathaniel; Crooks, Jean; Rueger, David C.; Ripamonti, Ugo [Reprint author]
CORPORATE SOURCE: Bone Research Laboratory, Medical School, MRC/University of the Witwatersrand, 7 York Road, Parktown, 2193, Johannesburg, South Africa
SOURCE: European Journal of Oral Sciences, (Oct., 1999) Vol. 107, No. 5, pp. 368-377. print.
ISSN: 0909-8836.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Dec 1999
Last Updated on STN: 3 Dec 1999

L8 ANSWER 9 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Articular cartilage **regeneration** by **OP**-1 (**BMP**-7) in sheep.
ACCESSION NUMBER: 1999:334935 BIOSIS
DOCUMENT NUMBER: PREV199900334935
TITLE: Articular cartilage **regeneration** by **OP**-1 (**BMP**-7) in sheep.
AUTHOR(S): Jelic, M. [Reprint author]; Haspi, M.; Kos, J.; Pecina, M.; Vukicevic, S. [Reprint author]
CORPORATE SOURCE: Department of Anatomy, School of Medicine, Zagreb, Croatia
SOURCE: Calcified Tissue International, (1999) Vol. 64, No. SUPPL. 1, pp. S93. print.
Meeting Info.: XXVIth European Symposium on Calcified Tissues. Maastricht, Netherlands. May 7-11, 1999. European Calcified Tissue Society.
CODEN: CTINDZ. ISSN: 0171-967X.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Aug 1999
Last Updated on STN: 24 Aug 1999

L8 ANSWER 10 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Osteogenic protein (**OP**-1, **BMP**-7) stimulates cartilage differentiation of human and goat perichondrium tissue in vitro.
AB The objective of this study was to examine in vitro the influence of recombinant human osteogenic protein-1 (rhOP-1, or bone morphogenetic protein-7 (**BMP**-7)) on cartilage formation by human and goat perichondrium tissue containing progenitor cells with chondrogenic potential. Fragments of outer ear perichondrium tissue were embedded in clotting autologous blood to which rhOP-1 had been added or not added (controls), and the resulting explant was cultured for 3 weeks without further addition of rhOP-1. Cartilage formation was monitored

biochemically by measuring (35S)-sulphate incorporation into proteoglycans and histologically by monitoring the presence of metachromatic matrix with cells in nests. The presence of rhOP-1 in the explant at the beginning of culture stimulated (35S)sulphate incorporation into proteoglycans in a dose-dependent manner after 3 weeks of culture. Maximal stimulation was reached at 40 mug/mL (human explants: +148%; goat explants: +116%). Histology revealed that explants treated with 20-200 mug/mL of rhOP-1, but not untreated control explants, contained areas of metachromatic-staining matrix with chondrocytes in cell nests. It was concluded that rhOP-1 stimulates differentiation of cartilage from perichondrium tissue. The direct actions of rhOP-1 on perichondrium cells in the stimulation of chondrocytic differentiation and production of cartilage matrix in vitro provides a cellular mechanism for the induction of cartilage formation by rhOP-1 in vivo. Thus rhOP-1 may promote early steps in the cascade of events leading to cartilage formation and could prove to be an interesting factor in the **regeneration** of cartilage in articular cartilage defects.

ACCESSION NUMBER: 1998:252548 BIOSIS
DOCUMENT NUMBER: PREV199800252548
TITLE: Osteogenic protein (**OP**-1, **BMP**-7) stimulates cartilage differentiation of human and goat perichondrium tissue in vitro.
AUTHOR(S): Klein-Nulend, J. [Reprint author]; Louwerse, R. T.; Heyligers, I. C.; Wuisman, P. I. J. M.; Semeins, C. M.; Goei, S. W.; Burger, E. H.
CORPORATE SOURCE: ACTA-Vrije Univ., Dep. Oral Cell Biol., Van der Boechorststraat 7, 1081 BT Amsterdam, Netherlands
SOURCE: Journal of Biomedical Materials Research, (June 15, 1998) Vol. 40, No. 4, pp. 614-620. print.
CODEN: JBMRBG. ISSN: 0021-9304.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 9 Jun 1998
Last Updated on STN: 9 Jun 1998

L8 ANSWER 11 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Recombinant transforming growth factor-beta-1 induces endochondral bone in the baboon and synergizes with recombinant osteogenic protein-1 (bone morphogenetic protein-7) to initiate rapid bone formation.
AB Several members of the bone morphogenetic protein (**BMP**) and transforming growth factor-beta (TGF-beta families are molecular regulators of cartilage and bone **regeneration**, although their actual roles and combined interactions in skeletal repair are poorly understood. The presence of several molecular forms suggests multiple functions in vivo as well as synergistic interactions during both embryonic bone development and **regeneration** of cartilage and bone in postfetal life. Here we show for the first time that recombinant human transforming growth factor-beta-1 (TGF-beta-1) induces endochondral bone formation in extraskeletal sites of adult baboons. We also show that TGF-beta-1 and recombinant human osteogenic protein-1 (**OP**-1, bone morphogenetic protein-7) synergize in inducing large ossicles in extraskeletal sites of the primate as early as 15 days after implantation. A single application of **OP**-1, in conjunction with an insoluble collagenous matrix as carrier (5, 25, and 125 mu-g/100 mg of carrier matrix) induced bone differentiation in the rectus abdominis of the baboon. This level of tissue induction was raised several-fold by the simultaneous addition of comparatively low doses of TGF-beta-1 (0.5, 1.5, and 5 Mg), which by itself induces bone formation in the rectus abdominis at doses of 5 mu-g/100 mg of carrier matrix. Combinations of **OP** -1 and TGF-beta-1 yielded a 2- to 3-fold increase in cross-sectional area of the newly generated ossicles, with markedly elevated key parameters of bone formation, and corticalization of the newly formed bone by day 15, culminating in bone marrow generation by day 30. The tissue generated by the combined application of **OP**-1 and TGF-beta-1 showed distinct

morphological differences when compared with **OP**-1-treated specimens, with large zones of endochondral development and extensive bone marrow formation. At the doses tested, synergy was optimal at a ratio of 1:20 by weight of TGF-beta-1 and **OP**-1, respectively. These results provide evidence for a novel function of TGF-beta-1 in the primate and the scientific basis for synergistic molecular therapeutics for the rapid **regeneration** of cartilage and bone.

ACCESSION NUMBER: 1997:496969 BIOSIS
DOCUMENT NUMBER: PREV199799796172
TITLE: Recombinant transforming growth factor-beta-1 induces endochondral bone in the baboon and synergizes with recombinant osteogenic protein-1 (bone morphogenetic protein-7) to initiate rapid bone formation.
AUTHOR(S): Qipamonti, U. [Reprint author]; Duneas, N.; Van Den Heever, B.; Bosch, C.; Crooks, J.
CORPORATE SOURCE: Bone Res. Lab., MRC/Univ. Witwatersrand Med. Sch., 7 York Road, Parktown 2193, Johannesburg, South Africa
SOURCE: Journal of Bone and Mineral Research, (1997) Vol. 12, No. 10, pp. 1584-1595.
CODEN: JBMREJ. ISSN: 0884-0431.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Nov 1997
Last Updated on STN: 21 Nov 1997

L8 ANSWER 12 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI **Regeneration** of articular cartilage defects in rabbits by osteogenic protein-1 (bone morphogenetic protein-7).
AB Osteogenic protein-1 (**OP**-1, **BMP**-7), a member of the transforming growth factor-beta family, induces cartilage and bone formation when implanted at intra and extraskeletal sites in vivo. The human **OP**-1 gene has been cloned and biologically active recombinant **OP**-1 homodimers have been produced. In the present study, the authors investigated the influence of **OP**-1 on healing of full-thickness articular cartilage defects, made by drilling two adjacent (vphi 3 mm) holes through articular cartilage of NZW rabbit knee joint. Defects were filled with collagen type I gel containing **OP** -1 or gel alone. Animals were killed after eight weeks, and knee joints were dissected and examined histomorphometrically. Results indicated that **OP**-1 induced articular cartilage healing and **regeneration** of the joint surface which contained cells resembling mature joint chondrocytes. These data imply a new strategy for biological repair of damaged joint surfaces in humans.

Non-articular claimed

ACCESSION NUMBER: 1997:244801 BIOSIS
DOCUMENT NUMBER: PREV199799544004
TITLE: **Regeneration** of articular cartilage defects in rabbits by osteogenic protein-1 (bone morphogenetic protein-7).
AUTHOR(S): Grgic, Marko; Jelic, Mislav; Basic, Vanja; Basic, Nikolina; Pecina, Marko; Vukicevic, Slobodan [Reprint author]
CORPORATE SOURCE: Dr. Drago Perovic Inst. Anatomy, Sch. Med., Univ. Zagreb, Salata 11, 10000 Zagreb, Croatia
SOURCE: Acta Medica Croatica, (1997) Vol. 51, No. 1, pp. 23-27.
ISSN: 1330-0164.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Jun 1997
Last Updated on STN: 13 Jun 1997

L8 ANSWER 13 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Markers of bone and cementum formation accumulate in tissues regenerated in periodontal defects treated with expanded polytetrafluoroethylene membranes.
AB Guided tissue **regeneration** (GTR) is a concept that evolved from

the development of membrane-barrier techniques, which allow the repopulation of periodontal wounds by specific cells, resulting in a new attachment apparatus. To help understand the biological mechanisms involved in membrane barrier led periodontal healing, the present study investigated the macromolecules phenotypic of bone and cementum formation in tissues grown under the GTR barrier by immunolocalization. Periodontal **regeneration** was initiated by placing barriers on experimentally induced periodontal defects in a Rhesus monkey model. Samples were harvested 6 wk after healing and sections of soft tissues grown under GTR barriers (membrane tissue) were stained with antibodies to bone morphogenetic proteins-2 and 4 (**BMP**-2, **BMP**-4), bone morphogenetic protein-7 (**OP**-1), cementum attachment protein (CAP), osteonectin (OTN) and bone sialoprotein (BSP). Tissues grown in the absence of any barrier device served as a control (control tissue). Membrane periodontal tissues from beneath the ePTFE membrane were comprised of spindle-shaped fibroblast-like cells encased in a dense fibrillar extracellular matrix (ECM). Round-shaped cells aggregated to form nodules. Newly formed hard tissue was conspicuous. A similar, but very disorganized, fiber network was observed in control tissues, but neither nodule formation nor hard tissue was observed. Osteonectin staining was observed in the ECM of membrane tissues and particularly in the area of the connective tissue adjacent to newly formed hard tissue. The dense network of connective tissue fibers was also stained. In control tissues, cells and fiber network had a significantly weaker signal for osteonectin. An intense reaction was observed in membrane tissues stained for BSP, particularly the connective tissue adjacent to the newly formed hard tissue, while the control tissues did not stain for BSP. Cementum attachment protein (CAP) was observed in the connective tissue adjacent to the newly formed hard tissue of the membrane tissues whereas control tissues exhibited no CAP staining. In membrane tissues, **BMP**-2 and 4 distribution was found to concentrate in nodule areas, in the newly formed hard tissue and in the fiber network, while very faint staining was observed in control sections. The distribution of **OP** -1 in membrane and control tissues was found to mimic the **BMP**-2 pattern, but staining was more distributed in hard tissue matrix. When the profile of **BMP**-2, **BMP**-4, **OP**-1, OTN, CAP and BSP staining was analyzed on membrane tissue sections, striking similarities were noted in the connective tissue adjacent to the newly formed hard tissue and in nodular areas. In addition, the localization of **BMP**-2 and **BMP**-4 mRNA was investigated in both tissues by in situ hybridization. An intense expression of **BMP**-2 and 4 transcripts was observed in membrane tissues while control tissues never yielded any positive hybridization signal. The correlation between these histochemical findings strongly suggests that the forming soft tissues under ePTFE membranes contain cells and ECM macromolecules normally associated with bone and cementum.

ACCESSION NUMBER: 1997:201209 BIOSIS
DOCUMENT NUMBER: PREV199799500412
TITLE: Markers of bone and cementum formation accumulate in tissues regenerated in periodontal defects treated with expanded polytetrafluoroethylene membranes.
AUTHOR(S): Amar, S. [Reprint author]; Chung, K. M.; Nam, S. H.; Karatzas, S.; Myokai, F.; Van Dyke, T. E.
CORPORATE SOURCE: Boston Univ., Sch. Dent. Med., Dep. Oral Biol. and Periodontol., W-201-C, 700 Albany St., Boston, MA 02118, USA
SOURCE: Journal of Periodontal Research, (1997) Vol. 32, No. 1 PART 2, pp. 148-158.
CODEN: JPDRAY. ISSN: 0022-3484.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 12 May 1997
Last Updated on STN: 12 May 1997

L8 ANSWER 14 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Regulation of neural cell adhesion molecule and L1 by the transforming growth factor-beta superfamily: Selective effects of the bone morphogenetic proteins.
AB The transforming growth factor-beta (TGF-beta) superfamily plays a role in embryogenesis and **regeneration**. We have reported that osteogenic protein-1 (**OP**-1) promotes cell aggregation and induces the expression of the neural cell adhesion molecules N-CAM and L1 in proliferating neuroblastoma times glioma hybrid NG108-15 cells (Perides, G., Safran, R. M., Rueger, D. C., and Charness, M. E. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10326-10330; Perides, G., Hu, G., Rueger, D. C., and Charness, M. E. (1993) J. Biol. Chemical 268, 25197-25205). Here we show that the structurally homologous bone morphogenetic proteins (**BMP**) **BMP**-2 and **BMP**-4 are 10-50-fold more potent in these actions than the subfamily comprising **BMP**-5, **BMP**-6, and **OP**-1 (**BMP**-7). In contrast, members of the TGF-beta subfamily, activin-A, inhibin-A, and 29 additional growth factors and cytokines did not induce N-CAM. The addition of serum to cells growing in serum-free medium caused a concentration-dependent increase in N-CAM and L1 expression; however, serum did not potentiate the induction of N-CAM and L1 by 40 ng(ml **OP**-1. These findings suggest the presence in NG108-15 cells of a **BMP**-2/**BMP**-4 receptor that discriminates subtle differences in structure among homologous members of the TGF-beta superfamily. An endogenous ligand for this receptor may be present in serum.
ACCESSION NUMBER: 1994:123943 BIOSIS
DOCUMENT NUMBER: PREV199497136943
TITLE: Regulation of neural cell adhesion molecule and L1 by the transforming growth factor-beta superfamily: Selective effects of the bone morphogenetic proteins.
AUTHOR(S): Perides, George; Safran, Rebecca M.; Downing, Lou Ann; Charness, Michael E. [Reprint author]
CORPORATE SOURCE: Dep Neurol. 127, Harvard Med. Sch., 1400 VFW Parkway, Boston, MA 02132, USA
SOURCE: Journal of Biological Chemistry, (1994) Vol. 269, No. 1, pp. 765-770.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Mar 1994
Last Updated on STN: 24 Mar 1994

L8 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI BOVINE OSTEOGENIC PROTEIN IS COMPOSED OF DIMERS OF **OP**-1 AND **BMP**-2A TWO MEMBERS OF THE TRANSFORMING GROWTH FACTOR-BETA SUPERFAMILY.
AB A bone-inductive protein has been purified from bovine bone and designated as osteogenic protein (**OP**). The purified **OP** induces new bone at less than 5 ng with half-maximal bone differentiation activity at about 20 ng/25 mg of matrix implant in a subcutaneous bone induction assay. The purified osteogenic protein is composed of disulfide-linked dimers that migrate on sodium dodecyl sulfate gels as a diffuse band with an apparent molecular weight of 30,000. Upon reduction, the dimers yield two subunits that migrate with molecular weights of 18,000 and 16,000. Both subunits are glycosylated. After chemical or enzymatic deglycosylation, the dimers migrate as a diffuse 27-kDa band that upon reduction yields two polypeptides that migrate at 16 kDa and 14 kDa, respectively. The carbohydrate moiety does not appear to be essential for biological activity since the deglycosylated proteins are capable of inducing bone formation in vivo. Amino acid sequences of peptides generated by proteolytic digestion show that the subunits are distinct but related members of the transforming growth factor-β superfamily. The 18-kDa subunit is the protein product of the bovine equivalent of the

human **OP**-1 gene and the 16-kDa subunit is the protein product of the bovine equivalent of the human **BMP**-2A gene.

ACCESSION NUMBER: 1990:444790 BIOSIS
DOCUMENT NUMBER: PREV199090095430; BA90:95430
TITLE: BOVINE OSTEOGENIC PROTEIN IS COMPOSED OF DIMERS OF **OP**-1 AND **BMP**-2A TWO MEMBERS OF THE TRANSFORMING GROWTH FACTOR-BETA SUPERFAMILY.
AUTHOR(S): SAMPATH T K [Reprint author]; COUGHLIN J E; WHETSTONE R M; BANACH D; CORBETT C; RIDGE R J; OZKAYNAK E; OPPERMANN H; RUEGER D C
CORPORATE SOURCE: CREATIVE BIO MOL INC, HOPKINTON, MASS 01748, USA
SOURCE: Journal of Biological Chemistry, (1990) Vol. 265, No. 22, pp. 13198-13205.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 7 Oct 1990
Last Updated on STN: 7 Oct 1990

=> s DPP
L9 1648 DPP

=> s Vgr-1
49 VGR
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L10 20 VGR-1
(VGR(W)1)

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L11 ANSWER 1 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Morphogen treatment of organ implants.
AB The present invention is directed to methods and compositions for enhancing viability of organs and living tissues to be transplanted in a mammal. The methods and compositions provide a therapeutically effective concentration of a morphogen or morphogen-stimulating agent to the tissue or organ to be transplanted, sufficient to substantially protect the tissue or organ from tissue damage.
ACCESSION NUMBER: 2001:181463 BIOSIS
DOCUMENT NUMBER: PREV200100181463
TITLE: Morphogen treatment of organ implants.
AUTHOR(S): Kuberasampath, Thangavel [Inventor]; Pang, Roy H. L. [Inventor]; Oppermann, Hermann [Inventor]; Rueger, David C. [Inventor]; Cohen, Charles M. [Inventor]; Smart, John E. [Inventor]
CORPORATE SOURCE: ASSIGNEE: Creative Bio Molecules, Inc., Boston, MA, USA
PATENT INFORMATION: US 6090776 July 18, 2000
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 18, 2000) Vol. 1236, No. 3. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 11 Apr 2001
Last Updated on STN: 18 Feb 2002

L11 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Bone morphogenetic proteins (BMP).
ACCESSION NUMBER: 1997:375092 BIOSIS
DOCUMENT NUMBER: PREV199799674295

TITLE: Bone morphogenetic proteins (BMP).
AUTHOR(S): Lacombe, D.
CORPORATE SOURCE: Clin. Pediatr. Genet. Med., Hop. Enfants, CHU Pellegrin, Pl. Amelie-Raba-Leon, 33076 Bordeaux, France
SOURCE: Archives de Pediatrie, (1997) Vol. 4, No. SUPPL. 2, pp. 121S-124S.
Meeting Info.: Spring Meeting of the Societe Francaise de Pediatrie (French Society of Pediatrics). Bordeaux, France. April 17-19, 1997.
ISSN: 0929-693X.
DOCUMENT TYPE: Conference; (Meeting Paper)
LANGUAGE: French
ENTRY DATE: Entered STN: 4 Sep 1997
Last Updated on STN: 27 Oct 1997

L11 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI OP-1 COMPLEMENTARY DNA ENCODES AN OSTEOGENIC PROTEIN IN THE TGF-BETA FAMILY.
AB Amino acid sequences of two tryptic peptides derived from enriched bovine osteogenic protein preparations revealed considerable homology to two members of the TGF-β (transforming growth factor β) supergene family, **DPP** (decapentaplegic protein) of Drosophila and Vg-1 (vegetal protein) of Xenopus. Building upon this information we constructed a synthetic consensus gene to use as a probe to screen human genomic libraries. This resulted in the isolation of three interrelated genes. Among these were BMP-2b and BMP-3 which have recently been described by others. The third gene, termed OP-1 (osteogenic protein one), is new and was subsequently shown to encode the human homolog of a major component of bovine osteogenic protein. The genomic clones were used to isolate the corresponding complementary DNA (cDNA) clones. Sequence analysis indicates that OP-1 is a relative of the murine **Vgr-1** (Vg-1 related gene). This report describes the cDNA structure and putative amino acid sequence of OP-1.
ACCESSION NUMBER: 1990:375460 BIOSIS
DOCUMENT NUMBER: PREV199090062141; BA90:62141
TITLE: OP-1 COMPLEMENTARY DNA ENCODES AN OSTEOGENIC PROTEIN IN THE TGF-BETA FAMILY.
AUTHOR(S): OZKAYNAK E [Reprint author]; RUEGER D C; DRIER E A; CORBETT C; RIDGE R J; SAMPATH T K; OPPERMANN H
CORPORATE SOURCE: CREATIVE BIOMOLECULES INC, 35 SOUTH STREET, HOPKINTON, MASS 01748, USA
SOURCE: EMBO (European Molecular Biology Organization) Journal, (1990) Vol. 9, No. 7, pp. 2085-2094.
CODEN: EMJODG. ISSN: 0261-4189.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 21 Aug 1990
Last Updated on STN: 21 Aug 1990

L11 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI **VGR-1** A MAMMALIAN GENE RELATED TO XENOPUS VG-1 IS A MEMBER OF THE TRANSFORMING GROWTH FACTOR BETA GENE SUPERFAMILY.
AB The transforming growth factor β (TGF-β)-related products of the Xenopus Vg-1 and Drosophila decapentaplegic (**DPP**) genes have been implicated in the control of growth and differentiation during embryogenesis. We have isolated a mouse cDNA, **Vgr-1**, that encodes a polypeptide structurally related to Xenopus Vg-1. Sequence comparisons indicate that the **Vgr-1** protein belongs to a family of **DPP**-like gene products within the TGF-β superfamily. The levels of **Vgr-1** RNA were determined in embryos and tissues isolated at various stages of development. A 3.5-kilobase mRNA increases throughout development and into adulthood in many tissues and in F9 teratocarcinoma cells differentiating into endoderm

in response to retinoic acid and cAMP. The amino acid homologies and patterns of expression suggest that, like the **DPP** gene product, **Vgr-1** plays a role at various stages of development.

ACCESSION NUMBER: 1989:357177 BIOSIS
DOCUMENT NUMBER: PREV198988049291; BA88:49291
TITLE: **VGR-1** A MAMMALIAN GENE RELATED TO XENOPUS VG-1 IS A MEMBER OF THE TRANSFORMING GROWTH FACTOR BETA GENE SUPERFAMILY.
AUTHOR(S): LYONS K [Reprint author]; GRAYCAR J L; LEE A; HASHMI S; LINDQUIST P B; CHEN E Y; HOGAN B L M; DERYNCK R
CORPORATE SOURCE: DEP CELL BIOL, VANDERBILT UNIV MED SCH, NASHVILLE, TENN 37203, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1989) Vol. 86, No. 12, pp. 4554-4558.
CODEN: PNASA6. ISSN: 0027-8424.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 2 Aug 1989
Last Updated on STN: 5 Aug 1989

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(FILE 'HOME' ENTERED AT 16:18:42 ON 31 MAR 2004)

FILE 'BIOSIS' ENTERED AT 16:21:50 ON 31 MAR 2004
L1 65221 S NONARTICULAR CARTILAGE ADJ REPAIR OR REGENERATION
L2 0 S L1 AND DEFECT LOCU
L3 0 S L1 AND DEFECT LOCUS
L4 4 S DEFECT LOCUS
L5 0 S L4 AND REPAIR
L6 0 S L4 AND CARTILAGE REPAIR
L7 144 S L1 AND BMP
L8 15 S L7 AND OP
L9 1648 S DPP
L10 20 S VGR-1
L11 4 S L10 AND L9

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L12 0 L1 AND L10

Refine Search

Search Results -

Terms	Documents
L1 and L8	16

Database:
US Pre-Grant Publication Full-Text Database
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DATE: Wednesday, March 31, 2004 Printable Copy Create Case

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L9	l1 and L8	16	L9
L8	l6 and L7	23	L8
L7	BMP	2412	L7
L6	protein and L5	146	L6
L5	gene and L4	186	L5
L4	"60 A"	4081	L4
L3	l1 and L2	0	L3
L2	vukicevic.in.	1	L2
L1	OP-1 or GDF3 or VGR-3	382	L1

END OF SEARCH HISTORY

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L9: Entry 4 of 16 File: USPT Apr 3, 2001

DOCUMENT-IDENTIFIER: US 6211146 B1
TITLE: 60A protein-induced morphogenesis

Abstract Text (1):
Disclosed are methods of utilizing a morphogenically active fragment of 60A protein to induce tissue morphogenesis, including methods for increasing a progenitor cell population in a mammal, methods for stimulating progenitor cells to differentiate and maintain their differentiated phenotype in vivo or in vitro, methods for inducing tissue-specific growth in vivo and methods for the replacement of diseased or damaged tissue in vivo.

Brief Summary Text (2):
This invention relates to methods for the use of morphogenically active fragments of 60A protein to induce tissue morphogenesis in mammals, including methods for promoting tissue stasis, repair and regeneration, and methods for increasing progenitor cell populations using morphogenically active fragments of the protein.

Brief Summary Text (6):
Recently, various members of the structurally related proteins of the transforming growth factor (TGF)-.beta. superfamily of proteins have been identified as true morphogens.

Brief Summary Text (7):
This "family" of proteins, sharing substantial amino acid sequence homology within their morphogenically active C-terminal domains, including a conserved six or seven cysteine skeleton, are capable of inducing tissue-specific morphogenesis in a variety of organs and tissues, including bone, cartilage, liver, dentin, periodontal ligament, cementum, nerve tissue and the epithelial mucosa of the gastrointestinal tract (see the copending, related U.S. applications Ser. No. 667,274, filed Mar. 11, 1991 abandoned in favor of (U.S. application Ser. No. 08/404,113 filed, Mar. 14, 1995) and U.S. application Ser. No. 08/404,113 filed Mar. 14, 1995 and U.S. application Ser. No. 08/260,675, filed Jun. 16, 1994 and U.S. application Ser. No. 08/132,883 filed May 21, 1995. The proteins apparently bind to surface receptors or otherwise contact and interact with progenitor cells, predisposing or stimulating the cells to proliferate and differentiate in a morphogenically permissive environment. The morphogens are capable of inducing the developmental cascade of cellular and molecular events that culminate in the formation of new organ-specific tissue, including any vascularization, connective tissue formation, and nerve ennervation as required by the naturally occurring tissue.

Brief Summary Text (8):
Among the proteins useful in tissue morphogenesis are proteins originally identified as bone inductive proteins, such as the OP-1 (comprising, e.g. Seq. ID NO. 3), OP-2 (comprising, e.g. Seq. ID NO. 5) and the CBMP2 (comprising, e.g. Seq. ID NO. 7 and 8) proteins, as well as amino acid sequence-related proteins such as BMP5 (comprising, e.g., Seq. ID NO. 14) and BMP6 (comprising, e.g., Seq. ID NO. 15), DPP (comprising, e.g., Seq. ID NO. 9) (from Drosophila), Vgl (comprising, e.g., Seq. ID NO. 10) (from Xenopus), and Vgr-1 (comprising, e.g., Seq. ID NO. 11) and GDF-1 (comprising, e.g., Seq. ID NO. 12) (from mouse see, for example,

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application Ser. No. 08/404,113, filed Mar. 14, 1995, U.S. Ser. No. 667,274 (abandoned in favor of CIP U.S. application Ser. No. 08/404,113, filed Mar. 14, 1995 and application Ser. No. 08/432,883 filed May 2, 1995. These TGF-.beta. superfamily members comprise a distinct subfamily of proteins different from other members of the TGF-.beta. superfamily in that the family of morphogenic proteins are able to induce the full cascade of events that result in tissue morphogenesis, including stimulating cell proliferation and cell differentiation, supporting the growth and maintenance of differentiated cells and inducing the "redifferentiation" of transformed cells to display a morphology characteristic of untransformed cells. The morphogenic proteins apparently act as endocrine factors rather than as local-acting growth factors like TGF-.beta.. Specifically, the endogenous morphogens may be synthesized and secreted from a factor-producing tissue and can be transported to, and act on, a tissue at a distance, e.g., a tissue other than the tissue in which they are synthesized.

Brief Summary Text (9):
The morphogens are synthesized in the cell as a precursor molecule approximately three times larger than the mature protein that is processed to yield mature disulfide-linked dimers comprising the C-terminal domain of the precursor sequence. The proteins are inactive when reduced, but are active as oxidized homodimeric species as well as when oxidized in combination with other morphogens to produce heterodimers. The proteins useful in tissue morphogenesis typically require a suitable environment enabling cells to proliferate and differentiate in a tissue-specific manner into, e.g., bone-producing osteoblasts, hemopoietic cells, or liver cells, depending on the nature of the local environment. The proliferation and differentiation of cells induced by the morphogenic proteins requires a suitable local environment including a suitable substratum on which the cells can anchor. The proliferating and differentiating cells also require the presence of appropriate signals to direct their tissue-specificity, such as cell surface markers.

Brief Summary Text (10):
Recently, another member of the TFG-.beta. superfamily of structurally related proteins has been identified in Drosophila melanogaster, the Drosophila 60A gene (comprising, e.g., Seq. ID NO. 1) (Wharton et al., (1991) Proc. Nat'l. Acad. Sci. USA 88: 9214-9218.) Northern blot analysis of Drosophila tissue with a C-terminal 60A-specific probe suggests that the 60A gene (comprising, e.g., Seq. ID NO. 1) is expressed throughout development with peaks of transcription during early embryogenesis. The 60A gene (comprising, e.g., Seq. ID NO. 1) consists of a single exon comprising a 1365 base-pair open reading frame encoding a 455 amino acid protein. It has been discovered that the encoded amino acid sequence for 60A (comprising, e.g., Seq. ID NO. 2) includes regions sharing high sequence homology with members of the family of morphogenic proteins.

Brief Summary Text (11):
It is an object of this invention to provide methods for utilizing a morphogenically active fragment of the 60A protein (comprising, e.g., Seq. ID NO. 2) to induce the developmental cascade of tissue morphogenesis for a variety of tissues in mammals. The morphogenic properties of 60A protein (comprising, e.g., Seq. ID NO. 2) include the ability to induce proliferation and differentiation of progenitor cells, and the ability to support and maintain the differentiated phenotype through the progression of events that results in the formation of adult tissue. Another object is to provide methods for the expression and isolation of a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) using recombinant DNA techniques. Still another object is to provide tissue-specific acellular matrices that may be used in combination with a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2), and methods for their production. Other objects include utilizing a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) in a range of methods, including methods for increasing a progenitor cell population in a mammal; methods for

stimulating progenitor cells to differentiate in vivo or in vitro and to maintain their differentiated phenotype; methods for inducing tissue-specific growth in vivo, and methods for the replacement of diseased or damaged tissue in vivo. These and other objects and features of the invention will be apparent from the description, drawings, and claims which follow.

Brief Summary Text (13):
This invention provides methods for utilizing morphogenically active fragments of 60A protein (comprising, e.g., Seq. ID NO. 2) to induce the developmental cascade of tissue morphogenesis in a mammal. Specifically, methods are provided for utilizing a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) to induce the proliferation of uncommitted progenitor cells, to induce the differentiation of these stimulated progenitor cells in a tissue-specific manner under appropriate environmental conditions, and to support the growth and maintenance of these differentiated cells. The protein also may be used to stimulate the "redifferentiation" of cells induced to stray from their differentiated phenotypes. Accordingly, 60A protein (comprising, e.g., Seq. ID NO. 2) can be utilized to initiate and maintain the developmental cascade of tissue morphogenesis in an appropriate, morphogenically permissive environment.

Brief Summary Text (14):
As used herein, useful Protein 60A morphogens include proteins encoded by the DNA sequence provided in Seq. ID No. 1 and allelic variants thereof, as well as other naturally-occurring and biosynthetic mutants that are morphogenically active as defined herein. "Morphogenically active fragments" is understood to include all proteins and protein fragments encoded by part or all of the sequence of Seq. ID No. 1, and which have morphogenic activity as defined herein. Specifically, as defined herein, a morphogen is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain comprises at least the C-terminal six cysteine skeleton defined by residues 359-455 of Seq. ID No. 1 (or residues 43-139 of Seq. ID No. 3), including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain disulfide bonds such that the protein is capable of acting as a morphogen as defined herein. Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. In addition, it is also anticipated that these morphogens are capable of inducing redifferentiation of committed cells under appropriate environmental conditions.

Brief Summary Text (16):
In one aspect of the invention, morphogenic 60A proteins (comprising, e.g., Seq. ID NO. 2) are useful in the replacement of diseased or damaged tissue in a mammal, such as damaged lung tissue resulting from emphysema; cirrhotic kidney or liver tissue; damaged heart or blood vessel tissue, as may result from cardiomyopathies and/or atherothrombotic or cardioembolic strokes; damaged stomach and other tissues of the gastrointestinal tract resulting from ulceric perforations or their repair; damaged nerve tissue as may result from physical injury, degenerative diseases such as Alzheimer's disease or multiple sclerosis, or strokes; damaged bone tissue as may result from metabolic bone diseases and other bone remodeling disorders; or damaged dentin, periodontal and/or cementum tissue as may result from disease or mechanical injury.

Brief Summary Text (17):

As provided herein, morphogenically active fragments of 60A protein (comprising, e.g., Seq. ID NO. 2) are provided to a tissue-specific locus in vivo, to induce the developmental cascade of tissue morphogenesis at that site. Cells stimulated ex vivo by contact with 60A protein (comprising, e.g., Seq. ID NO. 2) also may be provided to the tissue locus. In these cases the existing tissue provides the necessary matrix requirements, providing a suitable substratum for the proliferating and differentiating cells in a morphogenically permissive environment, as well as providing the necessary signals for directing the tissue-specificity of the developing tissue. Alternatively, the proteins or stimulated cells may be combined with a formulated matrix and implanted as a device at a locus in vivo. The formulated matrix should be a biocompatible, preferably biodegradable, appropriately modified tissue-specific acellular matrix having the characteristics described below.

Brief Summary Text (18):
In many instances, the loss of tissue function results from the tissue destructive effects and the subsequent formation of scar tissue associated with the body's immune/inflammatory response to an initial or repeated injury to the tissue. The degree of scar tissue formation generally depends on the regenerative properties of the injured tissue, and on the degree and type of tissue damage. Thus, in another aspect, morphogenically active fragments of 60A protein (comprising, e.g., Seq. ID NO. 2) may be used to prevent or to substantially inhibit the formation of scar tissue, including alleviating immune response-mediated tissue damage, by providing morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2) or cells stimulated by the 60A protein (comprising, e.g., Seq. ID NO. 2), to a newly injured tissue locus. The protein also may be provided as a prophylactic, provided to a site in anticipation of tissue injury, such as part of a surgical or other clinical procedure likely to produce tissue damage, and to induce an inflammatory/immune response.

Brief Summary Text (19):
60A protein (comprising, e.g., Seq. ID NO. 2) also may be used to increase or regenerate a progenitor or stem cell population in a mammal. For example, progenitor cells may be isolated from an individual's bone marrow, stimulated ex vivo with morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2) for a time and at a concentration sufficient to induce the cells to proliferate, and returned to the bone marrow. Other sources of progenitor cells that may be suitable include biocompatible cells obtained from a cultured cell line, stimulated in culture, and subsequently provided to the body. Alternatively, 60A protein (comprising, e.g., Seq. ID NO. 2) may be provided by systemic (e.g., oral or parenteral) administration, or it may be injected or otherwise provided to a progenitor cell population in an individual to induce its mitogenic activity in vivo. For example, a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) may be provided to the cells in vivo, e.g., by systemic injection, to induce mitogenic activity. Similarly, a particular population of hemopoietic stem cells may be increased by exposure to 60A protein (comprising, e.g., Seq. ID NO. 2), for example by plasmaphoresis of an individual's blood to extract the cells of interest, stimulating these cells ex vivo, and returning the stimulated cells to the blood.

Brief Summary Text (21):
In another aspect of the invention, morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2) may be used to support the growth and maintenance of differentiated cells, inducing existing differentiated cells to continue expressing their phenotype. It is anticipated that this activity will be particularly useful in the treatment of tissue disorders where loss of function is caused by reduced or lost metabolic function and cells become senescent or quiescent, such as may occur in aging cells and/or may be manifested in osteoporosis and a number of nerve degenerative diseases, including Alzheimer's disease. Application of 60A protein (comprising, e.g., Seq. ID NO. 2) directly to the cells to be treated, or providing

it systemically, as by oral or parenteral administration, can stimulate these cells to continue expressing their phenotype, thereby significantly reversing the effects of the dysfunction. In addition, a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) also may be used in gene therapy protocols to stimulate the growth of quiescent cells, thereby potentially enhancing the ability of these cells to incorporate exogenous DNA.

Brief Summary Text (22):
In yet another aspect of the invention, a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) also may be used to induce "redifferentiation" of cells that have strayed from their differentiation pathway, such as can occur during tumorgenesis. It is anticipated that this activity will be particularly useful in treatments to reduce or substantially inhibit the growth of neoplasms. The method also is anticipated to induce the de- and re-differentiation of these cells. As described supra, a morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2) fragment may be provided to the cells directly or systemically.

Brief Summary Text (23):
In still another aspect of the invention, 60A protein (comprising, e.g., Seq. ID NO. 2) may be used to stimulate cell adhesion molecule (CAM) expression levels in a cell. CAMs are molecules defined as carrying out cell-cell interactions necessary for tissue formation. CAMs are believed to play a fundamental regulatory role in tissue development, including tissue boundary formation, embryonic induction and migration, and tissue stabilization and regeneration. Altered CAM levels have been implicated in a number of tissue disorders, including congenital defects, neoplasias, and degenerative diseases.

Brief Summary Text (26):
The matrices utilized in the methods of the invention may be derived from organ-specific tissue, or they may be formulated synthetically. In one embodiment of the invention, when 60A protein (comprising, e.g., Seq. ID NO. 2) (or a collection of progenitor cells stimulated by 60A protein (comprising, e.g., Seq. ID NO. 2)) is provided at a tissue-specific locus, e.g., by systemic administration, implantation or injection at a tissue-specific locus, the existing tissue at that locus, whether diseased or damaged, has the capacity of acting as a suitable matrix. Alternatively, a formulated matrix may be provided externally together with the stimulated progenitor cells or morphogenically active 60A protein (comprising, e.g., Seq. ID NO. 2) fragment, as may be necessary when the extent of injury sustained by the damaged tissue is large. The matrix should be a biocompatible, suitably modified acellular matrix having dimensions such that it allows the influx, differentiation, and proliferation of migratory progenitor cells, and is capable of providing a morphogenically permissive environment. The matrix also preferably is tissue-specific, and biodegradable.

Brief Summary Text (28):
The invention thus relates to compositions and methods for the use of morphogenically active fragments of 60A protein, a species variant of the generic family of morphogens disclosed in U.S. Ser. No. 667,274 (abandoned in favor of CIP U.S. application Ser. No. 08/404,113, filed Mar. 14, 1995) and U.S. Ser. No. 752,764, (application Ser. No. 08/404,113, filed Mar. 14, 1995) as a tissue morphogen. Morphogenically active 60A proteins (comprising, e.g., Seq. ID NO. 2) and protein fragments can be isolated from naturally-occurring sources, or they may be constructed biosynthetically using conventional recombinant DNA technology. Active 60A protein (comprising, e.g., Seq. ID NO. 2) useful in the compositions and methods of this invention may include forms having varying glycosylation patterns, varying N-termini and active truncated forms, e.g., produced by recombinant DNA techniques. The 60A proteins (comprising, e.g., Seq. ID NO. 2) can be expressed from intact or truncated cDNA or from synthetic DNAs in procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Useful host cells include procaryotes, including E. coli, and

eucaryotic cells, including mammalian cells, such as CHO, COS or BSC cells, or the insect/baculovirus system. Thus recombinant DNA techniques may be utilized to produce large quantities of 60A protein (comprising, e.g., Seq. ID NO. 2) capable of inducing tissue-specific cell differentiation and tissue morphogenesis in a variety of mammals, including humans.

Brief Summary Text (30):
The invention provides methods and compositions for inducing the developmental cascade of tissue morphogenesis in a mammal utilizing morphogenically active fragments of the Drosophila melanogaster 60A protein (comprising, e.g., Seq. ID NO. 2). The methods and compositions provided herein may be utilized in a range of applications, including stimulating the proliferation and/or differentiation of progenitor cells and inducing the repair and regeneration of damaged tissue. The morphogenic 60A proteins (comprising, e.g., Seq. ID NO. 2) of the invention are a species variant of the family of morphogens disclosed in copending U.S. Ser. No. 667,274, U.S. Ser. No. 752,764, (abandoned in favor of CIP U.S. application Ser. No. 08/404,113, filed Mar. 14, 1995) application Ser. No. 08/404,113 filed Mar. 14, 1995) U.S. Ser. No. 923,780 and application Ser. No. 08/260,675, filed May 2, 1995 U.S. Ser. No. 922,813 application Ser. No. 08/260,675, filed Jun. 16, 1994 the disclosures of which are incorporated hereinabove by reference. As described herein, 60A protein (comprising, e.g., Seq. ID NO. 2) may be isolated from natural sources or constructed biosynthetically utilizing conventional recombinant DNA technology.

Brief Summary Text (31):
Morphogenically active fragments of 60A protein (comprising, e.g., Seq. ID NO. 2) are useful for initiating and maintaining the tissue-specific developmental cascade in a variety of tissues, including bone, cartilage, dentin, neural tissue, liver, periodontal ligament, cementum, lung, heart, kidney and numerous tissues of the gastrointestinal tract. When combined with naive progenitor cells as disclosed herein, morphogenically active 60A proteins (comprising, e.g., Seq. ID NO. 2) can induce the proliferation and differentiation of these progenitor cells. In the presence of appropriate tissue-specific signals to direct the differentiation of these cells, and a morphogenically permissive environment, 60A proteins (comprising, e.g., Seq. ID NO. 2) are capable of reproducing the cascade of cellular and molecular events that occur during embryonic development to yield functional tissue. For example, the protein can induce the de novo formation of cartilage and endochondral bone, including inducing the proliferation and differentiation of progenitor cells into chondrocytes and osteoblasts, inducing appropriate mineralization and bone remodeling, inducing formation of an appropriate bone tissue vascular supply and inducing formation of differentiated bone marrow (see Example 7 below.)

Brief Summary Text (32):
Provided below is a detailed description of the 60A proteins (comprising, e.g., Seq. ID NO. 2) useful in the compositions and methods of this invention, a description of how to make them, and methods and means for their therapeutic administration. Also provided are numerous, nonlimiting examples which (1) illustrate the suitability of these proteins as tissue morphogens and therapeutic agents, and (2) provide assays with which to test the morphogens encompassed by the invention in different tissues.

Brief Summary Text (34):
As defined herein a protein is morphogenic if it is capable of inducing the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue and comprises at least the conserved C-terminal six cysteine skeleton or its functional equivalent (see supra). Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor

cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. Details of how the morphogen family of proteins described herein first were identified, as well as a description of how to make, use and test them for morphogenic activity are disclosed in U.S. Ser. No. 667,274, (abandoned in favor of CIP U.S. application Ser. No. 08/404,113, filed Mar. 14, 1995) filed Mar. 11, 1991 and U.S. Ser. No. 752,764, application Ser. No. 08/404,113, filed Mar. 14, 1995 filed Aug. 30, 1991. As disclosed therein, the morphogens may be purified from naturally-sourced material or recombinantly produced from procaryotic or eucaryotic host cells, preferably as described therein, using the genetic sequences disclosed therein. Alternatively, novel morphogenic sequences may be identified following the procedures disclosed therein.

Brief Summary Text (35):
A candidate morphogen or morphogen composition can be evaluated for in vivo morphogenic utility generally according to the procedures set forth in U.S. application Ser. No. 08/404,113, filed Mar. 14, 1995. The proteins and compositions may be injected or surgically implanted in a mammal, following any of a number of procedures well known in the art. For example, surgical implant bioassays may be performed essentially following the procedure of Sampath et al. (1983) PNAS 80:6591-6595.

Brief Summary Text (47):
Incorporation of systemically provided morphogens may be followed using tagged morphogens (e.g., radioactively labelled) and determining their localization in new tissue, and/or by monitoring their disappearance from the circulatory system using a standard pulse-chase labeling protocol. The morphogen also may be provided with a tissue-specific molecular tag, whose uptake may be monitored and correlated with the concentration of morphogen provided. As an example, ovary removal in female rats results in reduced bone alkaline phosphatase activity, rendering the rats predisposed to osteoporosis. If the female rats now are provided with a morphogen, e.g., OP-1, a reduction in the systemic concentration of calcium (CA.sup.2+) is seen, which correlates with the presence of the provided morphogen and can be shown to correspond to increased alkaline phosphatase activity.

Brief Summary Text (48):
Particularly useful proteins identified to date include OP1 (comprising e.g., Seq. ID NO. 3), OP2 (comprising, e.g., Seq. ID NO. 5), CBMP2A (comprising, e.g., Seq. ID NO. 7) and CBMP2B (comprising, e.g., Seq. ID NO. 8) the morphogenically active domains of proteins referred to in the art as BMP2A and BMP2B, or BMP2 and BMP4, respectively), BMP3 (comprising, e.g., Seq. ID NO. 3), BMP5 (comprised, e.g., Seq. ID NO. 14), BMP6 (comprising, e.g., Seq. ID NO. 15), GDF-1, (comprising, e.g., Seq. ID NO. 12) Vgl, Vgr-1, (comprising, e.g., Seq. ID NO. 12) and (comprising, e.g., Seq. ID NO. 11) DPP, including their allelic and species variants, as well as other mutant variants. Detailed descriptions of the proteins also may be found in, for example, U.S. Ser. No. 922,813 application Ser. No. 08/260,674, filed Jun. 16, 1994. Morphogenically active biosynthetic constructs such as those disclosed in U.S. Pat. No. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16) also are envisioned to be useful.

Brief Summary Text (49):
Recently, the gene encoding a novel member of the TGF-.beta. superfamily of structurally related proteins was identified in the Drosophila genome and named "60A". The cDNA sequence and encoded amino acid sequence ("60A protein") are described in Wharton et al. (1991) Proc. Natl. Acad. Sci. USA, 88:9214-9218, and in Seq. ID No. 1.

Brief Summary Text (50):
The Drosphila 60A gene (comprising, e.g., Seq. ID NO. 1) encodes a protein ("60A")

first expressed as an immature translation product that is 455 amino acids in length. This precursor form, referred to herein as the "prepro" form, (Seq. ID. No. 1, residues 1-455) includes an N-terminal signal peptide sequence, typically less than about 20 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The "pro" form of the protein includes the pro domain and the mature domain, and forms a soluble species that appears to be the primary form secreted from cultured mammalian cells. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne ((1986) Nucleic Acids Research 14:4683-4691).

Brief Summary Text (51):
By amino acid sequence homology with other, known morphogens, the pro domain likely is cleaved at residues 322-325 of Seq. ID NO. 1, which represent the canonical Arg-Xaa-Xaa-Arg (Seq. ID NO. 16) cleavage site, to yield a mature sequence 130 amino acids in length (Seq. ID No. 1, residues 326-455). Another, longer form of the mature sequence is defined by residues 281-455. All morphogens comprise at least a conserved six cysteine skeleton in the amino acid sequence C-terminal domain. The conserved six cysteine skeleton is defined in 60A protein (comprising, e.g., Seq. ID NO. 2) by residues 359-455; the conserved seven cysteine skeleton is defined by residues 354-455. The morphogenically active protein comprises a mature, processed sequence, including fragments thereof, appropriately dimerized and disulfide bonded.

Brief Summary Text (52):
The mature sequence of 60A protein (comprising, e.g., Seq. ID NO. 2) shares significant amino acid sequence homology with the morphogens identified to date. Specifically, the seven cysteine skeleton shows approximately 70% amino acid identity with the corresponding hOP1 sequence. The 60A protein (comprising, e.g., Seq. ID NO. 2) seven cysteine skeleton also shares approximately 73% amino acid identity with the corresponding sequences of Vg-1 , (comprising, e.g., Seq. ID NO. 10) Vgr-1 , (comprising, e.g., Seq. ID NO. 11) BMP5 (comprising, e.g., Seq. ID NO. 14) and BMP6 (comprising, e.g., Seq. ID NO. 15), and about 53% identity with the seven cysteine skeleton of CBMP2A (comprising, e.g., Seq. ID NO. 7) (BMP2) (comprising, e.g., Seq. ID NO. 8) and CBMP2B (BMP4). The 60A protein seven cysteine skeleton also shares about 59% identity with the corresponding sequence of another morphogen identified in Drosophila, DPP (comprising, e.g., Seq. ID NO. 9). Without being limited to a particular theory, based on amino acid homology, 60A protein (comprising, e.g., Seq. ID NO. 2) likely may be the Drosophila homolog or species variant of OP-1 (comprising, e.g., Seq. ID NO. 3).

Brief Summary Text (53):
Table I, set forth below, compares the C-terminal amino acid sequences defining the seven cysteine skeleton of 60A protein (comprising, e.g., Seq. ID NO. 2), native human OP-1 (hOP-1 Seq. ID No. 3), and DPP (from Drosophila), (Seq. ID. No. 9). In the table, three dots indicates that the amino acid in that position is the same as the amino acid in the corresponding position in 60A protein (comprising, e.g., Seq. ID NO. 2). In Table II the sequences of the morphogens OP2, (Seq. ID NO. 5) CBMP2A, (Seq. ID NO. 7) CBMP2B, (Seq. ID NO. 8) BMP3, (Seq. ID NO. 13) BMP5, (Seq. ID NO. 14) BMP6, Vgl, Vgr-1, (Seq. ID NO. 10) GDF-1, (Seq. ID NO. 3) DPP (Seq. ID NO. 9) and 60-A (Seq. ID NO. 2) all are compared to OP-1. (comprising, e.g., Seq. ID NO. 3) In both tables the sequences are aligned essentially following the method of Needleman et al. (1970) J. Mol. Biol., 48:443-453, calculated using the Align Program (DNAstar, Inc.) In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1 (comprising, e.g., ID NO. 3). Three dashes indicate that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A (comprising, e.g., Seq. ID NO. 7) and CBMP-2B (comprising, e.g., Seq. ID NO. 8) is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A (comprising, e.g., Seq. ID NO. 7) then comprising Lys and Ile, whereas CBMP-2B (comprising, e.g., Seq. ID NO. 8)

comprises Ser and Ile.

Brief Summary Text (54):
As is apparent from the foregoing amino acid sequence comparisons, significant amino acid changes can be made within the sequences while retaining the morphogenic activity. For example, while the GDF-1 (comprising, e.g., Seq. ID NO. 12) protein sequence depicted in Table II shares only about 50% amino acid identity with the hOP-1 (comprising, e.g., Seq. ID NO. 3) sequence described therein, the GDF-1 (comprising, e.g., Seq. ID NO. 12) sequence shares greater than 70% amino acid sequence homology (or "similarity") with the hOP-1 sequence (comprising, e.g., Seq. ID NO. 3), where "homology" or "similarity" includes allowed conservative amino acid changes within the sequence as defined by Dayoff, et al., Atlas of Protein Sequence and Structure vol.5, supp.3, pp.345-362, (M. O. Dayoff, ed., Nat'l BioMed. Res. Fd'n, Washington D.C. 1979.)

Brief Summary Text (55):
II. Formulations and Methods for Administering 60A Protein as Therapeutic Agents

Brief Summary Text (56):
II.A 60A Protein Considerations

Brief Summary Text (57):
The morphogens described herein may be provided to an individual by any suitable means, preferably directly or systemically, e.g., parenterally or orally. Where the morphogen is to be provided directly (e.g., locally, as by injection, to a desired tissue site), or parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the morphogen preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the morphogen thus may comprise normal physiologic saline (0.85% NaCl, 0.15M), pH 7-7.4. The aqueous solution containing the morphogen can be made, for example, by dissolving the protein in 50% ethanol, acetonitrile containing 0.1% trifluoroacetic acid (TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, to ten volumes of phosphate buffered saline (PBS), which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively. If desired, a given morphogen may be made more soluble by association with a suitable molecule. For example, the pro form of 60A protein (comprising, e.g., Seq. ID NO. 2) comprises a species that is soluble in physiological solutions. In fact, the endogenous protein is thought to be transported (e.g., secreted and circulated) to particular tissues in this form. This soluble form of the protein may be obtained from the culture medium of morphogen-secreting mammalian cells. Alternatively, a soluble species may be formulated by complexing the mature dimer (or an active fragment thereof) with part or all of a pro domain. Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 (comprising, e.g., Seq. ID NO. 3) by 80%. Other components found in milk and/or various serum proteins also may be useful.

Brief Summary Text (59):
Alternatively, the morphogens described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins readily are degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the morphogens described herein typically are acid-stable and protease-resistant (see, for example, U.S. Pat. No. 4,968,590.) In addition, at least one morphogen, OP-1, (comprising, e.g., Seq. ID NO. 3) has been identified in bovine mammary gland

extract, colostrum and milk, as well as saliva. Moreover, the OP-1 (comprising, e.g., Seq. ID NO. 3) purified from mammary gland extract is morphogenically active. For example, this protein induces endochondral bone formation in mammals when implanted subcutaneously in association with a suitable matrix material, using a standard in vivo bone assay, such as is disclosed in U.S. Pat. No. 4,968,590. In addition, endogenous morphogen also is detected in human serum. These findings indicate that oral and parenteral administration are viable means for administering morphogens to an individual. Moreover, while the mature forms of certain morphogens described herein typically are sparingly soluble, the morphogen form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, morphogenically active form with the pro domain of the intact sequence and/or by association with one or more milk components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo, including, for example, part or all of a morphogen pro domain, and casein, as described above.

Brief Summary Text (60):
The compounds provided herein also may be associated with molecules capable of targeting the morphogen to a desired tissue. For example, tetracycline and diphosphonates (bisphosphonates) are known to bind to bone mineral, particularly at zones of bone remodeling, when they are provided systemically in a mammal. Accordingly, these molecules may be included as useful agents for targeting 60A protein (comprising, e.g., Seq. ID NO. 2) to bone tissue. Alternatively, an antibody or other binding protein that interacts specifically with a surface molecule on the desired target tissue cells also may be used. Such targeting molecules further may be covalently associated to the morphogen with, for example, an acid labile bond such as an Asp-Pro linkage, using standard chemical means well known in the art. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

Brief Summary Text (61):
As described above, the morphogen family members share significant sequence homology in the C-terminal active domains. By contrast, the sequences diverge significantly in the sequences which define the pro domain. Accordingly, the pro domain may be morphogen-specific. As described above, it also is known that the various morphogens identified to date are differentially expressed in the different tissues. Accordingly, without being limited to any given theory, it is likely that, under natural conditions in the body, selected morphogens typically act on a given tissue. Accordingly, part or all of morphogen pro domains may serve as targeting molecules for the morphogens described herein. For example, the pro domains may interact specifically with one or more molecules at the target tissue to direct the morphogen associated with the pro domain to that tissue. Accordingly, another useful targeting molecule for targeting 60A protein (comprising, e.g., Seq. ID NO. 2) to bone tissue, for example, may include part or all of a morphogen pro domain, particularly part or all of the pro domains of OP-1, (comprising, e.g., Seq. ID NO. 3) BMP2 or BMP4, all of which proteins are found naturally associated with bone tissue (see, for example, U.S. Pat. No. 5,011,691). Alternatively, the pro domain of GDF-1 (comprising, e.g., Seq. ID NO. 12) may be used to target morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2) to nerve tissue, particularly brain tissue where GDF-1 (comprising, e.g., Seq. ID NO. 12) appears to be primarily expressed (see, for example, CRP070 and Lee(1991)PNAS:88:4250-4254, incorporated herein by reference). As described above, morphogen species comprising the pro domain may be obtained from the culture medium of morphogen-secreting mammalian cells. Alternatively, a tissue-targeting species may be formulated by complexing the mature dimer (or an active fragment thereof) with part or all of a pro domain.

Brief Summary Text (62):
Finally, the morphogenic 60A proteins (comprising, e.g., Seq. ID NO. 2) provided herein may be administered alone or in combination with other molecules known to

have a beneficial effect on tissue morphogenesis, including molecules capable of tissue repair and regeneration and/or inhibiting inflammation. Examples of useful cofactors for stimulating bone tissue growth in osteoporotic individuals, for example, include vitamin D.sub.3, calcitonin, prostaglandins, parathyroid hormone, dexamethasone, estrogen and IGF-I or IGF-II. Useful cofactors for nerve tissue repair and regneration may include nerve growth factors.

Brief Summary Text (64):
The compositions can be formulated for parenteral or oral administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which provide appropriate concentrations of a morphogenic 60A protein to target tissue for a time sufficient to induce morphogenesis, including particular steps thereof, as described above.

Brief Summary Text (65):
As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition will vary depending upon a number of factors, including the dosage of the drug to be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of drug to be administered also is likely to depend on such variables as the type and extent of tissue loss or defect, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound excipients, and its route of administration. In general terms, the compounds of this invention may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for parenteral administration. Typical dose ranges are from about 10 ng/kg to about 1 g/kg of body weight per day; a preferred dose range is from about 0.1 .mu.g/kg to 100 mg/kg of body weight per day. Optimally, the 60A protein (comprising, e.g., Seq. ID NO. 2) dosage given in all cases is between 1-100 .mu.g of protein per kilogram weight of the patient. Currently preferred dose ranges for local injection of soluble 60A protein (comprising, e.g., Seq. ID NO. 2) to target tissue are 0.1-50 .mu.g morphogen/injection. No obvious morphogen-induced pathological lesions are induced when mature morphogen (e.g., OP-1, 20 .mu.g) is administered daily to normal growing rats for 21 consecutive days. Moreover, 10 .mu.g systemic injections of morphogen (e.g., OP-1 (comprising, e.g., Seq. ID NO. 3)) injected daily for 10 days into normal newborn mice does not produce any gross abnormalities.

Brief Summary Text (67):
A morphogenically active fragment of 60A protein may be implanted surgically, dispersed in a biocompatible, preferably in vivo biodegradable matrix appropriately modified to provide a structure in which the 60A protein (comprising, e.g., Seq. ID NO. 2) may be dispersed and which allows the influx, differentiation and proliferation of migrating progenitor cells. The matrix also may provide signals capable of directing the tissue specificity of the differentiating cells, as well as providing a morphogenically permissive environment, being essentially free of growth inhibiting signals.

Brief Summary Text (88):
The morphogenically active fragments of 60A protein (comprising, e.g., Seq. ID NO. 2) described herein can be combined and dispersed in an appropriately modified tissue-specific matrix using any of the methods described below:

Brief Summary Text (92):
In this procedure, a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) in an acetonitrile trifluroacetic acid (ACN/TFA) solution is added to the carrier material. Samples are vigorously vortexed many times and then lyophilized.

Brief Summary Text (94):

A preparation of a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) in physiological saline also may be vortexed with the matrix and lyophilized to produce morphogenically active material.

Brief Summary Text (95):
Tissue morphogenesis requires a morphogenically permissive environment. Clearly, in fully-functioning healthy tissue that is not composed of a permanently renewing cell population, there must exist signals to prevent continued tissue growth. Thus, it is postulated that there exists a control mechanism, such as a feedback control mechanism, which regulates the control of cell growth and differentiation. In fact, it is known that both TGF-.beta., and MIS are capable of inhibiting cell growth when present at appropriate concentrations. In addition, using the bone model system it can be shown that osteogenic devices comprising a bone-derived carrier (matrix) that has been demineralized and guanidine-extracted to substantially remove the noncollagenous proteins does allow endochondral bone formation when implanted in association with an osteoinductive morphogen. If, however, the bone-derived carrier is not demineralized but rather is washed only in low salt, for example, induction of endochondral bone formation is inhibited, suggesting the presence of one or more inhibiting factors within the carrier.

Detailed Description Text (3):
Recombinant Production of Morphogenic 60A Protein

Detailed Description Text (4):
The 60A proteins (comprising, e.g., Seq. ID NO. 2) useful in the methods and compositions of this invention may be purified from natural sources or produced using standard recombinant methodology. General considerations for the recombinant production of morphogens are described in U.S. Ser. Nos. 667,274 (abandoned in favor of CIP U.S. application Ser. No. 08/404,113, filed Mar. 14, 1995 and 752,764, (application Ser. No. 08/404,113, filed Mar. 14, 1995 the disclosures of which are incorporated hereinabove by reference.

Detailed Description Text (5):
A currently preferred protocol for producing 60A protein (comprising, e.g., Seq. ID NO. 2) uses cloning plasmids with commercially available promoters and selection marker sequences in the Drosophila S2 cell line, a Drosophila melanogaster cell line derived from late embryonic stages, as described below. A detailed description of the protocol can be found in Panganiban et al., (1990) Mol. Cell. Biol. 10:2669-2677 the disclosure of which is incorporated herein by reference. Briefly, the full length Drosophila 60A cDNA clone was incorporated into an expression plasmid that contained a metallothionein promoter and leader sequence and co-transfected into a host S2 cell with a selection plasmid (e.g., containing the marker gene dihydrofolate reductase for selection and amplication.) The expression promotor and selection sequences may be obtained commercially from, for example, from Clontech, Inc., Palo Alto, or the ATCC, Rockville, Md. (e.g.,from plasmid #37148).

Detailed Description Text (6):
Transfected cells were grown in M3 medium supplemented with 12.5% fetal cal serum (FCS, Gibco Laboratories) to which 2.times.10.sup.-7 M methotrexate (MTX) was added 3 days post transfection. On day 7, 4.times.10.sup.6 cells from each transfection, together with 10.sup.6 gamma-irradiated S2 feeder cells, were plated with 2 ml of 0.3% Noble agar. MTX-resistant cells then were serially subcultured for 74 days, subcultured once again, and transferred to small tissue culture flasks containing 3 ml media for 60A protein (comprising, e.g., Seq. ID NO. 2) induction with 500 .mu.m CUSO.sub.4. The 60A protein (comprising, e.g., Seq. ID NO. 2) expressed in the S2 system cells is produced as a processed mature disulfide-linked dimer and secreted into the medium as a soluble protein.

Detailed Description Text (7):
The recombinantly produced 60A protein (comprising, e.g., Seq. ID NO. 2) then was

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purified from the medium using two chromatography steps: S-sepharose (Sigma Chemical Co., St. Louis) and reverse phase HPLC (e.g., Aldrich Chemical Co., Milwaukee). A typical purification utilized 50 ml of medium containing 5% fetal calf serum. The media was diluted with 2 volumes of 9 M urea, 20 mM MES, pH 7.0 and applied to a 10 ml column of S-sepharose equilibrated with 6 M urea, 20 mM MES, pH 7.0, containing 50 mM NaCl.sub.2. After washing with the equilibration buffer, step elution of bound protein was accomplished with the same buffer containing 100 and 300 mM NaCl.sub.2. The 300 mM NaCl fraction then was sequentially dialysed against water and 30% acetonitrile/0.1% trifluoroacetic acid, and finally subjected to C18 reverse phase HPLC. Fractions containing morphogen (e.g., 60A protein (comprising, e.g., Seq. ID NO. 2)) were determined by immunoblot analysis (using 60A-specific polyclonal antibody) and Coomassie staining. Immunoreactive fractions then were pooled and the purity of 60A protein (comprising, e.g., Seq. ID NO. 2) determined by standard gel scanning methods. The concentration of protein was estimated by scanning the Coomassie-stained protein band in the gel at 580 nm in reference to a known amount of standard bovine serum albumin protein.

Detailed Description Text (8):
The purified protein is a processed mature disulfide-linked homodimer. The identity of the purified protein was confirmed by N-terminal sequencing and Western blot analysis using 60A-specific antisera.

Detailed Description Text (9):
Drosophila DPP (comprising, e.g., Seq. ID NO. 9) also was cloned and purified as described for 60A protein (comprising, e.g., Seq. ID NO. 2). DPP (comprising, e.g., Seq. ID NO. 9) was expressed and secreted as a processed mature disulfide-linked dimer which then bound to and accumulated on the petri dish surface. The DPP protein (comprising, e.g., Seq. ID NO. 9) that bound to plates was extracted with 200 mM CaCl.sub.2 /1% Tween-20/20 mM MES buffer pH 7.2, and purified on an S-Sepharose and C-18 column as described for 60A protein (comprising, e.g., Seq. ID NO. 2).

Detailed Description Text (10):
The identification of mature DPP (comprising, e.g., Seq. ID NO. 9) and 60A protein (comprising, e.g., Seq. ID NO. 2) was made by (1) N-terminal amino acid sequence analysis, (2) Western blot analysis using morphogen specific antisera, and (3) SDS-PAGE analysis under non-reduced and reduced conditions. Because of the homology at the C-terminal domains, the reaction of DPP (comprising, e.g., Seq. ID NO. 9) and 60A protein (comprising, e.g., Seq. ID NO. 2) with BMP-2 (CBMP2A (comprising, e.g., Seq. ID NO. 7)) and OP-1, (comprising, e.g., Seq. ID NO. 3) using antisera specific for these morphogens, was examined. Western blot analysis indicates DPP (comprising, e.g., Seq. ID NO. 9) reacts to BMP-2 antisera alone, and 60A protein (comprising, e.g., Seq. ID NO. 2) reacts with OP-1-specific antisera alone. The morphogen-specific antisera employed in the Western blots were produced by immunizing rabbits with the C-terminal domains of E. coli produced human BMP-2 and BMP4, for BMP-2-specific antisera, or E. coli produced OP-1 (comprising, e.g., Seq. ID NO. 3) (for OP-1-specific antibody), using standard immunology techniques. The antibody cross-reactivity data, together with the significant amino acid sequence homology, further suggests that 60A protein (comprising, e.g., Seq. ID NO. 2) likely may be the Drosophila homolog or species variant of OP1, and DPP (comprising, e.g., Seq. ID NO. 9), the homolog or the species variant of BMP2.

Detailed Description Text (12):
Mitogenic Effect of 60A Protein

Detailed Description Text (14):
The ability of 60A protein (comprising, e.g., Seq. ID NO. 2) to induce proliferation of osteoblasts may be determined in vitro using the following assay. In this and all examples involving osteoblast cultures, rat osteoblast-enriched primary cultures preferably are used. Although these cultures are heterogeneous in

that the individual cells are at different stages of differentiation, the culture is believed to more accurately reflect the metabolism and function of osteoblasts in vivo than osteoblast cultures obtained from established cell lines. Unless otherwise indicated, all chemicals referenced are standard, commercially available reagents, readily available from a number of sources, including Sigma Chemical, Co., St. Louis; Calbiochem, Corp., San Diego and Aldrich Chemical Co., Milwaukee.

Detailed Description Text (16):
The cultured cells are divided into three groups: (1) wells which receive, for example, 0.1, 1.0, 10.0, 40 and 80.0 ng of 60A protein (comprising, e.g., Seq. ID NO. 2); (2) wells which receive 0.1, 1.0, 10.0 and 40 ng of a local-acting growth factor (e.g., TGF-.beta.); and (3) the control group, which receive no growth factors. The cells then are incubated for an additional 18 hours after which the wells were pulsed with 2 .mu.Ci/well of .sup.3 H-thymidine and incubated for six more hours. The excess label then is washed off with a cold solution of 0.15 M NaCl, and then 250 .mu.l of 10% tricholoracetic acid added to each well and the wells incubated at room temperature for 30 minutes. The cells then are washed three times with cold distilled water, and lysed by the addition of 250 .mu.l of 1% sodium dodecyl sulfate (SDS) for a period of 30 minutes at 37.degree. C. The resulting cell lysates are harvested using standard means well known in the art, and the incorporation of .sup.3 H-thymidine into cellular DNA determined by liquid scintillation as an indication of mitogenic activity of the cells. In the experiment, 60A protein (comprising, e.g., Seq. ID NO. 2) stimulates .sup.3 H-thymidine incorporation into DNA, and thus promote osteoblast cell proliferation. By contrast, the effect of TGF-.beta. is transient and biphasic. At high concentrations, TGF-.beta. has no significant effect on osteoblast cell proliferation.

Detailed Description Text (17):
The in vitro effect of 60A protein (comprising, e.g., Seq. ID NO. 2) on osteoblast proliferation also can be tested on human primary osteoblasts (obtained from bone tissue of a normal adult patient and prepared as described above) and on human osteosarcoma-derived cell lines. In all cases 60A protein is anticipated to induce cell proliferation in accordance with the morphogen's ability to induce endochondral bone formation (see Example 7, below).

Detailed Description Text (19):
The ability of 60A protein (comprising, e.g., Seq. ID NO. 2) to stimulate the proliferation of progenitor cells also can be assayed readily in vitro using the following assay. Useful naive stem cells include pluripotential stem cells, which may be isolated from bone marrow or umbilical cord blood using conventional methodologies, (see, for example, Faradji et al., (1988) Vox Sanq., 55 (3):133-138 or Broxmeyer et al., (1989) PNAS 86:3828-3832), as well as naive stem cells obtained from blood. Alternatively, embryonic cells (e.g., from a cultured mesodermal cell line) may be useful.

Detailed Description Text (20):
Another method for obtaining progenitor cells and for determining the ability of 60A protein (comprising, e.g., Seq. ID NO. 2) fragments to stimulate cell proliferation is to capture progenitor cells from an in vivo source. For example, a biocompatible matrix material able to allow the influx of migratory progenitor cells may be implanted at an in vivo site long enough to allow the influx of migratory progenitor cells. For example, a bone-derived, guanidine-extracted matrix, formulated as disclosed for example in Sampath et al. ((1983) PNAS 80:6591-6595), or U.S. Pat. No. 4,975,526, may be implanted into a rat at a subcutaneous site, essentially following the method of Sampath et al. After three days the implant is removed, and the progenitor cells associated with the matrix dispersed and cultured.

Detailed Description Text (21):

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Progenitor cells, however obtained, then are incubated in vitro with a morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2) fragment under standard cell culture conditions well described in the art and described hereinabove. In the absence of external stimuli, the progenitor cells do not, or only minimally, proliferate on their own in culture. However, progenitor cells cultured in the presence of a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) do proliferate. Cell growth can be determined visually or spectrophotometrically using standard methods well known in the art.

Detailed Description Text (25):
Morphogenically active fragments of 60A protein (comprising, e.g., Seq. ID NO. 2) can be utilized to induce cell differentiation. The ability of 60A protein (comprising, e.g., Seq. ID NO. 2) to induce cell differentiation can be determined by culturing early mesenchymal cells in the presence of 60A protein (comprising, e.g., Seq. ID NO. 2) and then studying the histology of the cultured cells by staining with toluidine blue using standard cell culturing and cell staining methodologies well described in the art. For example, it is known that rat mesenchymal cells destined to become mandibular bone, when separated from the overlying epithelial cells at stage 11 and cultured in vitro under standard tissue culture conditions, e.g., in a chemically defined, serum-free medium, containing for example, 67% DMEM (Dulbecco's modified Eagle's medium), 22% F-12 medium, 10 mM Hepes pH 7, 2 mM glutamine, 50 .mu.g/ml transferring, 25 .mu.g/ml insulin, trace elements, 2 mg/ml bovine serum albumin coupled to oleic acid, with HAT (0.1 mM hypoxanthine, 10 .mu.M aminopterin, 12 .mu.M thymidine, will not continue to differentiate. However, if these same cells are left in contact with the overlying endoderm for an additional day, at which time they become stage 12 cells, they will continue to differentiate on their own in vitro to form chondrocytes. Further differentiation into obsteoblasts and, ultimately, mandibular bone, requires an appropriate local environment, e.g., a vascularized environment.

Detailed Description Text (26):
Stage 11 mesenchymal cells, cultured in vitro in the presence of 60A protein (comprising, e.g., Seq. ID NO. 2), e.g., 10-100 .mu.g/ml, will continue to differentiate in vitro to form chondrocytes just as they continue to differentiate in vitro if they are cultured with the cell products harvested from the overlying endodermal cells. This experiment may be performed with different mesenchymal cells to assess the cell differentiation capability of different morphogenically active fragments of 60A protein (comprising, e.g., Seq. ID NO. 2).

Detailed Description Text (27):
As another example of morphogen-induced cell differentiation, the ability of 60A proteins (comprising, e.g., Seq. ID NO. 2) to induce osteoblast differentiation may be evaluated in vitro using primary osteoblast cultures or osteoblast-like cells lines and assaying for a variety of-bone cell markers that are specific markers for the differentiated osteoblast phenotype, e.g., alkaline phosphatase activity, parathyroid hormone-mediated cyclic AMP (cAMP) production, osteocalcin synthesis, and enhanced mineralization rates.

Detailed Description Text (28):
3.2 Alkaline Phosphatase Induction of Osteoblasts by 60A Protein

Detailed Description Text (29):
The cultured cells in serum-free medium are incubated with, for example, 0.1, 1.0, 10.0, 40.0 or 80.0 ng 60A protein (comprising, e.g., Seq. ID NO. 2)/ml medium; or TGF-.beta. at 0.1, 1.0, 10.0, 40.0 or 80.0 ng/ml medium for 72 hours. After the incubation period the cell layer is extracted with 0.5 ml of 1% Triton X-100. The resultant cell extract is centrifuged, 100 .mu.l of the extract is added to 90 .mu.l of paranitrosophenylphosphate (PNPP)/glycerine mixture and incubated for 30 minutes in a 37.degree. C. water bath and the reaction stopped with 100 .mu.l NaOH. The samples then are run through a plate reader (e.g., Dynatech MR700 plate

reader, and absorbance measured at 400 nm, using p-nitrophenol as a standard) to determine the presence and amount of alkaline phosphate activity. Protein concentrations are determined by the Biorad method. Alkaline phosphatase activity is calculated in units/.mu.g protein, where 1 unit=1 nmol p-nitrophenol liberated/30 minutes at 37.degree. C.

Detailed Description Text (30):
60A protein (comprising, e.g., Seq. ID NO. 2) alone stimulates the production of alkaline phosphatase in osteoblasts, and thus promotes the growth and expression of the osteoblast differentiated phenotype.

Detailed Description Text (32):
Rat osteoblasts are prepared and cultured in multi-well plates as described above. In this example six sets of 24 well plates are plated with 50,000 rat osteoblasts per well. The wells in each plate, prepared as described above, then are divided into three groups: (1) those which receive, for example, 1 ng of 60A protein (comprising, e.g., Seq. ID NO. 2) per ml of medium; (2) those which receive 40 ng of 60A protein (comprising, e.g., Seq. ID NO. 2) per ml of medium; and (3) those which received 80 ng of 60A protein (comprising, e.g., Seq. ID NO. 2) per ml of medium. Each plate then is incubated for different lengths of time: 0 hours (control time), 24 hours, 48 hours, 96 hours, 120 hours and 144 hours. After each incubation period, the cell layer is extracted with 0.5 ml of 1% Triton X-100. The resultant cell extract is centrifuged, and alkaline phospatase activity determined as for Example 3.1, using paranitroso-phenylphosphate (PNPP). 60A protein (comprising, e.g., Seq. ID NO. 2) stimulates the production of alkaline phosphatase in osteoblasts in dose-dependent manner so that increasing doses of 60A (comprising, e.g., Seq. ID NO. 2) further increase the level of alkaline phosphatase production, and moreover, the 60A-stimulated elevated levels of alkaline phosphatase in the treated osteoblasts is anticipated to last for an extended period of time.

Detailed Description Text (33):
3.3 60A Protein Induction of PTH-Mediated cAMP

Detailed Description Text (35):
Rat osteoblasts are prepared and cultured in a multiwell plate as described above. The cultured cells then are divided into three groups: (1) wells which receive, for example, 1.0, 10.0 and 40.0 ng 60A protein (comprising, e.g., Seq. ID NO. 2) ml medium); (2) wells which receive for example, TGF-.beta., at 0.1, 1.0, and 5.0 ng/ml medium); and (3) a control group which receives no growth factors. The plate is then incubated for another 72 hours. At the end of the 72 hours the cells are treated with medium containing 0.5% bovine serum albumin (BSA) and 1 mM 3-isobutyl-1-methylxanthine for 20 minutes followed by the addition into half of the wells of human recombinant parathyroid hormone (hPTH, Sigma, St. Louis) at a concentration of 200 ng/ml for 10 minutes. The cell layer then is extracted from each well with 0.5 ml of 1% Triton X-100. The cAMP levels then are determined using a radioimmunoassay kit (e.g., Amersham, Arlington Heights, Ill.). 60A protein (comprising, e.g., Seq. ID NO. 2) alone stimulates an increase in the PTH-mediated cAMP response, and thus promotes the growth and expression of the osteoblast differentiated phenotype.

Detailed Description Text (36):
3.4 60A Protein Induction of Osteocalcin Production

Detailed Description Text (37):
Osteocalcin is a bone-specific protein synthesized by osteoblasts which plays an integral role in the rate of bone mineralization in vivo. Circulating levels of osteocalcin in serum are used as a marker for osteoblast activity and bone formation in vivo. Induction of osteocalcin synthesis in osteoblast-enriched cultures can be used to demonstrate 60A (comprising, e.g., Seq. ID NO. 2)

morphogenic efficacy in vitro.

Detailed Description Text (38):
Rat osteoblasts are prepared and cultured in a multi-well plate as above. In this experiment the medium is supplemented with 10% FBS, and on day 2, cells are fed with fresh medium supplemented with fresh 10 mM .beta.-glycerophosphate (Sigma, Inc.). Beginning on day 5 and twice weekly thereafter, cells are fed with a complete mineralization medium containing all of the above components plus fresh L (+)-ascorbate, at a final concentration of 50 .mu.g/ml medium. 60A protein then is added to the wells directly, e.g., in 50% acetonitrile (or 50% ethanol) containing 0.1% trifluoroacetic acid (TFA), at no more than 5 .mu.l morphogen/ml medium. Control wells receive solvent vehicle only. The cells then are re-fed and the conditioned medium sample diluted 1:1 in standard radioimmunoassay buffer containing standard protease inhibitors and stored at -20.degree. C. until assayed for osteocalcin. Osteocalcin synthesis is measured by standard radioimmunoassay using a commercially available osteocalcin-specific antibody.

Detailed Description Text (40):
60A protein (comprising, e.g., Seq. ID NO. 2) stimulates osteocalcin synthesis in osteoblast cultures. The increased osteocalcin synthesis in response to 60A protein (comprising, e.g., Seq. ID NO. 2) is expected to be dose dependent and to show a significant increase over the basal level after 13 days of incubation. The enhanced osteocalcin synthesis also can be confirmed by detecting the elevated osteocalcin mRNA message (20-fold increase) using a rat osteocalcin-specific probe. In addition, the increase in osteoclacin synthesis is expected to correlate with increased mineralization in long term osteoblast cultures as determined by the appearance of mineral nodules 60A protein (comprising, e.g., Seq. ID NO. 2) is expected to increase the initial mineralization rate about 20-fold compared to untreated cultures.

Detailed Description Text (43):
The ability of 60A proteins (comprising, e.g., Seq. ID NO. 2) to stimulate CAM expression can be demonstrated using the following protocol, using NG108-15 cells. NG108-15 is a transformed hybrid cell line (neuroblastoma x glioma, America Type Tissue Culture (ATCC), Rockville, Md.), exhibiting a morphology characteristic of transformed embryonic neurons. As described in Example 4, below, untreated NG108-15 cells exhibit a fibroblastic, or minimally differentiated, morphology and express only the 180 and 140 isoforms of N-CAM normally associated with a developing cell. Following morphogen treatment these cells exhibit a morphology characteristic of adult neurons and express enhanced levels of all three N-CAM isoforms.

Detailed Description Text (44):
In this example NG108-15 cells are cultured for 4 days in the presence of increasing concentrations of 60A protein (comprising, e.g., Seq. ID NO. 2) using standard culturing procedures, and standard Western blots performed on whole cell extracts. N-CAM isoforms are detected with an antibody which crossreacts with all three isoforms, mAb H28.123, obtained from Sigma Chemical Co., St. Louis, the different isoforms being distinguishable by their different mobilities on an electrophoresis gel. Control NG108-15 cells (untreated) express both the 140 kDa and the 180 kDa isoforms, but not the 120 kDa, as determined by Western blot analyses using up to 100 .mu.g of protein. Treatment of NG108-15 cells with 60A protein (comprising, e.g., Seq. ID NO. 2) results in a dose-dependent increase in the expression of the 180 kDa and 140 kDa isoforms, as well as the induction of the 120 kDa isoform induced. In addition, CAM expression correlates with cell aggregation as well as morphology, as determined by histology. Morphogen treatment also induces expression of another neural CAM, L1.

Detailed Description Text (46):
60A Protein-Induced Redifferentiation of Transformed Phenotype

Detailed Description Text (47):
The 60A protein (comprising, e.g., Seq. ID NO. 2) morphogens described herein also can induce redifferentiation of transformed cells to a morphology characteristic of untransformed cells. The examples provided below detail morphogen-induced redifferentiation of a transformed human cell line of neuronal origin (NG108-15); as well as mouse neuroblastoma cells (N1E-115), and human embryo carcinoma cells, to a morphology characteristic of untransformed cells.

Detailed Description Text (48):
As described above, NG108-15 is a transformed hybrid cell line produced by fusing neuroblastoma x glioma cells (obtained from ATTC, Rockville, Md.), and exhibiting a morphology characteristic of transformed embryonic neurons, e.g., having a fibroblastic morphology. Specifically, the cells have polygonal cell bodies, short, spike-like processes and make few contacts with neighboring cells (see copending U.S. Ser. No. 922,813 application Ser. No. 08/860,674, filed Jun. 16, 1994). Incubation of NG108-15 cells, cultured in a chemically defined, serum-free medium, with 0.1 to 300 ng/ml of morphogen (e.g; OP-1 (comprising, e.g., Seq. ID NO. 3)) for four hours induces an orderly, dose-dependent change in cell morphology.

Detailed Description Text (49):
In the example, NG108-15 cells are subcultured on poly-L-lysine coated 6 well plates. Each well contains 40-50,000 cells in 2.5 ml of chemically defined medium. On the third day, 2.5 .mu.l of morphogen (e.g., 60A protein (comprising, e.g., Seq. ID NO. 2)) in 60% ethanol containing 0.025% trifluoroacetic is added to each well. Morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2) of varying concentrations may be tested (typically, concentration ranges of 0-300 ng/ml are tested). The media is changed daily with new aliquots of morphogen. Morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2) induces a dose-dependent redifferentiation of the transformed cells, including a rounding of the soma, an increase in phase brightness, extension of the short neurite processes, and other significant changes in the cellular ultrastructure. After several days treated cells will begin to form epithelioid sheets that then become highly packed, multi-layered aggregates, as determined visually by microscopic examination.

Detailed Description Text (50):
Moreover, morphogen-induced redifferentiation occurs without any associated changes in DNA synthesis, cell division, or cell viability, making it unlikely that the morphologic changes are secondary to cell differentiation or a toxic effect of the morphogen. In addition, the morphogen-induced redifferentiation does not inhibit cell division, as determined by .sup.3 H-thymidine uptake, unlike other molecules which have been shown to stimulate differentiation of transformed cells, such as butyrate, DMSO, retanoic acid or Forskolin in analogous experiments. Thus, 60A protein (comprising, e.g., Seq. ID NO. 2) maintains cell stability and viability after inducing redifferentiation.

Detailed Description Text (51):
The 60A protein (comprising, e.g., Seq. ID NO. 2) morphogens described herein accordingly provide useful therapeutic agents for the treatment of neoplasias and neoplastic lesions of the nervous system, particularly in the treatment of neuroblastomas, including retinoblastomas, and gliomas.

Detailed Description Text (52):
As yet another, related example, the ability of 60A proteins (comprising, e.g., Seq. ID NO. 2) to induce the "redifferentiation" of transformed human cells may be demonstrated. Specifically, the effect of 60A protein (comprising, e.g., Seq. ID NO. 2) fragments on human EC cells (embryo carcinoma cells, e.g., NTERA-Z CL.D1, ATCC, Rockville, Md.) may be determined. In the absence of an external stimulant, these cells can be maintained as undifferentiated stem cells, and can be induced to grow in serum free media (SFM). In the absence of treatment with a morphogen, the cells proliferate rampantly and are anchorage-independent. In the presence of

morphogen EC cells grow as flattened cells, becoming anchorage dependent and forming aggregates. In addition, growth rate is reduced approximately 10 fold. Ultimately, the cells are induced to differentiate. In the example, varying concentrations of 60A protein (comprising, e.g., Seq. ID NO. 2) (e.g., 0-300 ng/ml) are added daily to cultured cells (e.g., 40-50,000 cells in 2.5 ml chemically defined medium), and the effects of treatment determined by visual examination. 60A protein (comprising, e.g., Seq. ID NO. 2) stimulates redifferentiation of these cells to a morphology characteristic of untransformed embryo cells.

Detailed Description Text (55):
Morphogenically active fragments of 60A protein (comprising, e.g., Seq. ID NO. 2) also may be used to maintain a cell's differentiated phenotype. This application is particularly useful for inducing the continued expression of phenotype in senescent or quiescent cells.

Detailed Description Text (57):
The phenotypic maintenance capability of morphogens is assessed readily. A number of differentiated cells become senescent or quiescent after multiple passages in vitro under standard tissue culture conditions such well described in the art, e.g., Culture of Animal Cells: A Manual of Basic Techniques (R. Freshney, ed., Wiley, 1987). However, if these cells are cultivated in vitro in association with a morphogen such as 60A protein (comprising, e.g., Seq. ID NO. 2), cells are stimulated to maintain expression of their phenotype through multiple passages. For example, the alkaline phosphatase activity of cultured osteoblasts, such as cultured osteosarcoma cells and calvaria cells, is significantly reduced after multiple passages in vitro. However, if the cells are cultivated in the presence of a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2), alkaline phosphatase activity is maintained over extended periods of time. Similarly, phenotypic expression of myocytes also is maintained in the presence of a morphogen. In the experiment, osteoblasts are cultured as described in Example 2. The cells are divided into groups, incubated with varying concentrations of 60A protein (comprising, e.g., Seq. ID NO. 2) (e.g., 0-300 ng/ml) and passaged multiple times (e.g., 3-5 times) using standard methodology. Passaged cells then are tested for alkaline phosphatase activity, as described in Example 3 as an indication of differentiated cell metabolic function. Osteoblasts cultured in the absence of 60A protein (comprising, e.g., Seq. ID NO. 2) have a reduced alkaline phosphatase activity, as compared to 60A protein-treated cells.

Detailed Description Text (59):
Phenotypic maintenance capability also may be assessed in vivo, using a rat model for osteoporosis, as disclosed in the copending U.S. Ser. No. 752,857, application Ser. No. 08/155,343, filed Nov. 15, 1993, filed Aug. 30, 1991, and [U.S. Ser. No. 923,780 application Ser. No. 08/432,883, filed May 2, 1995] incorporated herein above by reference. As described therein, Long Evans female rats (Charles River Laboratories, Wilmington, Mass.) are ovariectomized using standard surgical techniques, to produce an osteoporotic condition resulting from decreased estrogen production. Eight days after ovariectomy, rats are systemically provided with phosphate buffered saline (PBS) or morphogen, (e.g., 60A protein, 2-20 .mu.g) for 21 days. The rats then are sacrificed and serum alkaline phosphatase levels, serum calcium levels, and serum osteocalcin levels are determined, using standard methodologies as described therein and above. Elevated levels of osteocalcin and alkaline phosphatase are anticipated in the rats treated with an effective amount of a morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2). Moreover, histomorphometric analysis on the tibial diasypheal bone shows improved bone mass in 60A protein-treated animals as compared with untreated, ovariectomized rats. In fact, the bone mass of 60A protein-animals is comparable to that of the sham-operated (e.g., nonovarectomized) rats.

Detailed Description Text (62):
Progenitor cells may be stimulated to proliferate in vivo or ex vivo. The cells may

be stimulated in vivo by injecting or otherwise providing a sterile preparation containing the morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) into the individual. For example, the hemopoietic pluripotential stem cell population of an individual may be stimulated to proliferate by injecting or otherwise providing an appropriate concentration of the morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) to the individual's bone marrow.

Detailed Description Text (63):
Progenitor cells may be stimulated ex vivo by contacting progenitor cells of the population to be enhanced with a morphogenically active fragment of 60A protein under (comprising, e.g., Seq. ID NO. 2) sterile conditions at a concentration and for a time sufficient to stimulate proliferation of the cells. Suitable concentrations and stimulation times may be determined empirically, essentially following the procedure described in Example 2, above. A morphogen concentration of between about 0.1-100 ng/ml and a stimulation period of from about 10 minutes to about 72 hours, or, more generally, less than about 24 hours, typically should be sufficient to stimulate a cell population of about 10.sup.4 to 10.sup.6 cells. The stimulated cells then are provided to the individual as, for example, by injecting the cells to an appropriate in vivo locus. Suitable biocompatible progenitor cells may be obtained by any of the methods known in the art or described hereinabove.

Detailed Description Text (66):
The morphogenically active fragments of Drosophila 60A protein (comprising, e.g., Seq. ID NO. 2) may be used to repair diseased or damaged mammalian tissue. The tissue to be repaired preferably is assessed first, and excess necrotic or interfering scar tissue removed as needed, e.g., by ablation or by surgical, chemical or other methods known in the medical arts.

Detailed Description Text (67):
The 60A protein (comprising, e.g., Seq. ID NO. 2) then may be provided directly to the tissue locus as part of a sterile, biocompatible composition, either by surgical implantation or injection. The morphogen also may be provided systemically, as by oral or parenteral administration. Alternatively, a sterile, biocompatible composition containing progenitor cells stimulated by a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) may be provided to the tissue locus. The existing tissue at the locus, whether diseased or damaged, provides the appropriate matrix to allow the proliferation and tissue-specific differentiation of progenitor cells. In addition, a damaged or diseased tissue locus, particularly one that has been further assaulted by surgical means, provides a morphogenically permissive environment. Systemic provision of a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) will be sufficient for certain applications (e.g., in the treatment of osteoporosis and other disorders of the bone remodeling cycle, as an example).

Detailed Description Text (68):
In some circumstances, particularly where tissue damage is extensive, the tissue may not be capable of providing a sufficient matrix for cell influx and proliferation. In these instances, it may be necessary to provide the 60A protein (comprising, e.g., Seq. ID NO. 2) or progenitor cells stimulated by 60A protein (comprising, e.g., Seq. ID NO. 2) to the tissue locus in association with a suitable, biocompatible, formulated matrix, prepared by any of the means described below. The matrix preferably is in vivo biodegradable. The matrix also may be tissue-specific and may comprise porous particles having dimensions within the range of 70-850 .mu.m, most preferably 150-420 .mu.m.

Detailed Description Text (69):
The morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2) also may be used to prevent or substantially inhibit immune/inflammatory response mediated tissue damage and scar tissue formation following an injury. 60A protein (comprising,

e.g., Seq. ID NO. 2) is provided to a newly injured tissue locus, to induce tissue morphogenesis at the locus, preventing the aggregation of migrating fibroblasts into non-differentiated connective tissue. The 60A protein (comprising, e.g., Seq. ID NO. 2) fragment preferably is provided as a sterile pharmaceutical preparation injected into the tissue locus within five hours of the injury.

Detailed Description Text (70):
Below are several examples, describing protocols for assessing 60A protein-induced tissue morphogenesis in bone, liver, nerve, dentin, cementum and periodontal ligament.

Detailed Description Text (71):
7.1 60A Protein-Induced Bone Morphogenesis

Detailed Description Text (72):
A particularly useful mammalian tissue model system for demonstrating and evaluating the morphogenic activity of a protein is the endochondral bone tissue morphogenesis model known in the art and described, for example, in U.S. Pat. No. 4,968,590 and incorporated herein by reference. The ability to induce endochondral bone formation includes the ability to induce the proliferation and subsequent differentiation of progenitor cells into chondroblasts and osteoblasts, the ability to induce cartilage matrix formation, cartilage calcification, and bone remodeling, and the ability to induce formation of an appropriate vascular supply and hematopoeitic bone marrow differentiation.

Detailed Description Text (74):
The following sets forth various procedures for evaluating the in vivo morphogenic utility of the morphogenically active fragments of 60A protein (comprising, e.g., Seq. ID NO. 2) and compositions thereof. The fragments and compositions may be injected or surgically implanted in a mammal, following any of a number of procedures well known in the art. For example, surgical implant bioassays may be performed essentially following the procedure of Sampath et al. (1983) PNAS 80:6591-6595 and U.S. Pat. No. 4,968,590.

Detailed Description Text (78):
Incorporation of systemically provided morphogenic fragments of 60A protein (comprising, e.g., Seq. ID NO. 2) may be followed using tagged fragments (e.g., radioactively labelled) and determining their localization in the new tissue, and/or by monitoring their disappearance from the circulatory system using a standard labeling protocol and pulse-chase procedure. The 60A protein (comprising, e.g., Seq. ID NO. 2) also may be provided with a tissue-specific molecular tag, whose uptake may be monitored and correlated with the concentration of the 60A protein (comprising, e.g., Seq. ID NO. 2) fragment provided. As an example, ovary removal in female rats results in reduced bone alkaline phosphatase activity and renders the rats predisposed to osteoporosis (as described in Example 5). If the female rats now are provided with a 60A protein (comprising, e.g., Seq. ID NO. 2), a reduction in the systemic concentration of calcium may be seen, which correlates with the presence of the provided 60A protein (comprising, e.g., Seq. ID NO. 2) and which is anticipated to correspond with increased alkaline phosphatase activity.

Detailed Description Text (79):
In this example, the recombinantly produced mature disulfide-linked homodimers of two Drosophila morphogens, DPP (comprising, e.g., Seq. ID NO. 9) and 60A proteins (comprising, e.g., Seq. ID NO. 2), were evaluated for their capacity to induce new endochondral bone formation in mammals using the rat subcutaneous bone induction model. As disclosed herein, both Drosophila proteins, DPP (comprising, e.g., Seq. ID NO. 9) and 60A protein (comprising, e.g., Seq. ID NO. 2), can induce the formation of new cartilage, bone and bone marrow at non-bony sites in mammals.

Detailed Description Text (80):

Purified, recombinant DPP (comprising, e.g., Seq. ID NO. 9) and 60A gene (comprising, e.g., Seq. ID NO. 2) products, produced as described in Example 1, above, then were combined in varying concentrations with rat collagen carrier and assayed for bone forming activity in rats as described above and in the art.

Detailed Description Text (81):
Briefly, DPP (comprising, e.g., Seq. ID NO. 9) or 60A protein (comprising, e.g., Seq. ID NO. 2) was reconstituted with rat collagen carrier by the 50% acetonitrile/0.1% TFA lyophilization method as described. In this example, 25 mg of 4 M guanidine HCl-extracted demineralized rat collagenous matrix (rat collagen carrier) was added to varying concentrations of protein dissolved in 200 .mu.l of 50% acetonitrile/0.1% TFA, vortexed and then lyophilized. Rat collagen carrier alone was the negative control and intact demineralized bone matrix was the positive control. The day of implantation was designated as day 0 of the assay. Implants were removed on day 12 and bone forming activity in the implants was monitored by the specific activity of alkaline phosphatase and calcium content as described above. Values are the average of four to six observations from two to three rats. Table III evaluates the bone forming activity of 60A protein, DPP and OP. As can be seen, all three morphogens induce alkaline phosphatase activity and inherent calcium content.

Detailed Description Text (83):
Table III, below, catalogs the histology results of 60A protein (comprising, e.g., Seq. ID NO. 2) and DPP (comprising, e.g., Seq. ID NO. 9) induced endochondral bone formation. Bone formation was calculated as described in U.S. Pat. No. 4,968,590. Specifically, one bone forming unit represents the amount of protein needed for half maximal bone forming activity of the implant on day 12. The bone forming activity elicited by intact bone matrix is considered to be the maximal bone differentiation activity for comparison purposes in this assay. As is evident from the histology results, extensive bone formation and bone remodeling can be seen throughout the micrograph in a 60A protein (comprising, e.g., Seq. ID NO. 2) implant. Similar results were obtained using DPP implants, indicating that morphogens present in Drosophila induce de novo tissue regeneration in mammals.

Detailed Description Text (85):
As another example, a method for inducing morphogenesis of substantially injured liver tissue following a partial hepatectomy utilizing a morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2) is presented. Variations on this general protocol may be used to test morphogen activity of 60A protein (comprising, e.g., Seq. ID NO. 2) fragments in other different tissues. The general method involves excising an essentially nonregenerating portion of a tissue and providing the morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2), preferably as a soluble pharmaceutical preparation to the excised tissue locus, closing the wound, and examining the site at a future date. Like bone, liver has a potential to regenerate upon injury during post-fetal life.

Detailed Description Text (86):
The morphogenic 60A protein (comprising, e.g., Seq. ID NO. 2), e.g., 1 mg/ml, in a biocompatible solution, for example, (e.g., a purified recombinant mature form, is solubilized in 50% ethanol (or compatible solvent) containing 0.1% trifluoroacetic acid (or compatible acid). Alternatively, the mature protein may be solubilized by association with a pro domain. The injectable 60A protein (comprising, e.g., Seq. ID NO. 2) solution is prepared, e.g., by diluting one volume of 66A protein (comprising, e.g., Seq. ID NO. 2) solvent-acid stock solution with 9 volumes of 0.2% rat serum albumin in sterile PBS (phosphate-buffered saline).

Detailed Description Text (87):
In the experiment, growing rats or aged rats (e.g., Long Evans, Charles River Laboratories, Wilmington) are anesthetized by using ketamine. Two of the liver lobes (left and right) are cut out (approximately 1/3 of the lobe) and the 60A

protein (comprising, e.g., Seq. ID NO. 2) is injected locally at multiple sites along the cut ends. The amount of 60A protein (comprising, e.g., Seq. ID NO. 2) injected may be, e.g., 100 .mu.g in 1000 .mu.l of PBS/RSA (phosphate buffered saline/rat serum albumin) injection buffer. Placebo samples are injection buffer only. In experimental assays, five rats in each group preferably are used. The wound is closed and the rats are allowed to eat normal food and drink tap water.

Detailed Description Text (88):
After 12 days, the rats are sacrificed and liver regeneration is observed visually, to evaluate the effects of the 60A protein (comprising, e.g., Seq. ID NO. 2) on liver regeneration most effectively. The 60A protein fragment-injected group shows, e.g., complete liver tissue regeneration with no sign remaining of any cut in the liver. By contrast, the control group into which only PBS is injected shows only minimal regeneration with the incision remaining in the sample. Previous experiments with other morphogens (e.g., OP-1 (comprising, e.g., Seq. ID NO. 3)) show these morphogens alone induce liver tissue regeneration.

Detailed Description Text (90):
As still another example, the ability of a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) to induce dentinogenesis also may be demonstrated. To date, the unpredictable response of dental pulp tissue to injury is a basic clinical problem in dentistry. Cynomolgus monkeys are chosen as primate models as monkeys are presumed to be more indicative of human dental biology than models based on lower non-primate mammals.

Detailed Description Text (92):
Pulp treatments used may include: a morphogenically active fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) dispersed in a carrier matrix; carrier matrix alone, and no treatment. Twelve teeth per animal (four for each treatment) are prepared, and two animals are used. At four weeks, teeth are extracted and processed histologically for analysis of dentin formation, and/or ground to analyze dentin mineralization. The effect of a fragment of 60A protein (comprising, e.g., Seq. ID NO. 2) on osteodentin reparation may be observed visually by comparing control samples treatment (PBS) with 60A protein (comprising, e.g., Seq. ID NO. 2) or DPP (comprising, e.g., Seq. ID NO. 9). 60A protein (comprising, e.g., Seq. ID NO. 2) plus a carrier matrix induces formation of reparative or osteodentin bridges on surgically exposed healthy dental pulps. By contrast, pulps treated with carrier matrix alone, do not to form reparative dentin.

Detailed Description Text (93):
Similarly, implanting demineralized teeth and 60A protein (comprising, e.g., Seq. ID NO. 2) into surgically prepared canine tooth sockets are anticipated to stimulate cementum and periodontal ligament formation, as well as new bone tissue, as described in U.S. Ser. No. application Ser. No. 08/155,343, filed Nov. 15, 1993, filed herewith, the disclosure of which is incorporated herein by reference.

Detailed Description Text (95):
As yet another example, the induction of regenerative effects on central nervous system (CNS) repair, by a morphogenically active fragment of 60A protein, may be demonstrated using a rat brain stab model. Briefly, male Long Evans rats are anesthetized and the head area prepared for surgery. The calvariae is exposed using standard surgical procedures and a hole drilled toward the center of each lobe using a 0.035K wire, just piercing the calvariae. 25 .mu.l solutions containing either morphogen (e.g., 60A protein (comprising, e.g., Seq. ID NO. 2), 25 .mu.g in PBS) or PBS alone then is provided to each of the holes by Hamilton syringe. Solutions are delivered to a depth approximately 3 mm below the surface, into the underlying cortex, corpus callosum and hippocampus. The skin then is sutured and the animal allowed to recover.

Detailed Description Text (96):

Three days post surgery, rats are sacrificed by decapitation and their brains processed for sectioning. Scar tissue formation is evaluated by immunofluoresence staining for glial fibrillary acidic protein, a marker protein for glial scarring, to qualitatively determine the degree of scar formation. Sections also are probed with 60A protein-specific antibody to determine the presence of the protein. Reduced levels of glial fibrillary acidic protein are seen in the tissue sections of animals treated with 60A protein (comprising, e.g., Seq. ID NO. 2) evidencing the ability of the morphogen to inhibit glial scar formation, thereby stimulating nerve regeneration.

Detailed Description Text (97):
The ability of 60A protein (comprising, e.g., Seq. ID NO. 2) to stimulate peripheral nervous system axonal growth over extended distances may be demonstrated using the following model. Neurons of the peripheral nervous system can sprout new processes on their own following injury, but without guidance these sproutings typically fail to connect appropriately and die. Where the break is extensive, e.g., greater than 5 or 10 mm, regeneration is poor or nonexistent. Previous experiments with other morphogens, e.g., OP-1, (comprising, e.g., Seq. ID NO. 3) show that morphogens stimulate peripheral nervous system axonal growth over extended distances, allowing repair and regeneration of damaged peripheral neural pathways.

Detailed Description Text (98):
In this example 60A protein (comprising, e.g., Seq. ID NO. 2) stimulation of nerve regeneration is assessed using the rat sciatic nerve model. The rat sciatic nerve can regenerate spontaneously across a 5 mm gap, and occasionally across a 10 mm gap, provided that the severed ends are inserted in a saline-filled nerve guidance channel. In this experiment, nerve regeneration across at least a 12 mm gap is tested.

Detailed Description Text (99):
Adult female Sprague-Dawley rats (Charles River Laboratories, Inc.) weighing 230-250 g are anesthetized with intraperitoneal injections of sodium pentobarbital (35 mg/kg body weight). A skin incision is made parallel and just posterior to the femur. The avascular intermuscular plane between vastus lateralis and hamstring muscles are entered and followed to the loose fibroareolar tissue surrounding the sciatic nerve. The loose tissue is divided longitudinally thereby freeing the sciatic nerve over its full extent without devascularizing any portion. Under a surgical microscope the sciatic nerves are transected with microscissors at mid-thigh and grafted with a 60A protein (comprising, e.g., Seq. ID NO. 2) gel graft that separates the nerve stumps by 12 mm. The graft region is encased in a silicone tube 20 mm in length with a 1.5 mm inner diameter, the interior of which is filled with the morphogen solution. Specifically, The central 12 mm of the tube consists of an 60A protein (comprising, e.g., Seq. ID NO. 2) gel prepared by mixing 1 to 5 .mu.g of substantially pure recombinately produced 60A protein (comprising, e.g., Seq. ID NO. 2) with approximately 100 .mu.l of MATRIGEL.TM. (from Collaborative Research, Inc., Bedford, Mass.), an extracellular matrix extract derived from mouse sarcoma tissue, and containing solubilized tissue basement membrane, including laminin, type IV collagen, heparin sulfate, proteoglycan and entactin, in phosphate-buffered saline. The morphogen-filled tube then is implanted directly into the defect site, allowing 4 mm on each end to insert the nerve stumps. Each stump is abutted against the morphogen gel and is secured in the silicone tube by three stitches of commercially available surgical 10-0 nylon through the epineurium, the fascicle protective sheath.

Detailed Description Text (100):
In addition to 60A protein (comprising, e.g., Seq. ID NO. 2) gel grafts, control grafts of empty silicone tubes, silicone tubes filled with gel only and "reverse" autografts, wherein 12 mm transected segments of the animal's sciatic nerve are rotated 180.degree. prior to suturing, preferably also are grafted. All experiments

preferably are performed in quadruplicate. All wounds preferably are closed by wound clips that are removed after 10 days. Rats can be grafted on both legs. At 3 weeks the animals are sacrificed, and the grafted segments removed and frozen on dry ice immediately. Frozen sections then are cut throughout the graft site, and examined for axonal regeneration by immunofluorescent staining using anti-neurofilament antibodies labeled with flurocein (obtained, for example, from Sigma Chemical Co., St. Louis).

Detailed Description Text (101):
Regeneration of the sciatic nerve is anticipated to occur across the entire 12 mm distance in all graft sites wherein the gap is filled with the 60A protein (comprising, e.g., Seq. ID NO. 2) gel. By contrast, empty silicone tubes, gel alone and reverse autografts do not show nerve regeneration.

Detailed Description Paragraph Table (1):
TABLE III BONE INDUCING ACTIVITY BY RECOMBINANT DPP AND 60A Protein Calcium Concentration Alkaline Content (ng/implant) Phosphatase .mu.g/mg (25 mg) U/mg protein tissue Histology* (comprising, eq., -- 0.06 -- Seq. ID No. 9) 480 1.43 N.D +++ DPP 1440 1.48 N.D +++ 60A -- -- (comprising, eq, 400 N.D 9.75 ++ Seq ID No. 2) 800 N.D 15.20 +++ 1600 N.D 19.60 +++ *histology observed on day 12 ++ moderate bone formation +++ excellent bone formation N.D - Not determined

Other Reference Publication (25):
Wharton et al "Drosophila 60A Gene . . . " PNAS 88:9214-9218 (Oct. 1991).*

CLAIMS:

1. A composition comprising:

(a) a biocompatible, acellular matrix; and

(b) a protein comprising:

(i) the amino acid sequence of SEQ ID NO: 2; or

(ii) a naturally occurring allelic variant of (i); or

(iii) a fragment of (i) comprising a C-terminal six cysteine skeleton; or

(iv) a conservative amino acid substitution variant of any of (i)-(iii),

wherein said composition induces endochondral bone formation in an in vivo assay for bone formation.

2. The composition of claim 1 wherein said protein comprises amino acids 354 to 455 of SEQ ID NO: 2, or a conservative amino acid substitution variant thereof.

3. The composition of claim 1 wherein said protein comprises amino acids 326 to 455 of SEQ ID NO: 2, or a conservative amino acid substitution variant thereof.

4. The composition of claim 1 wherein said protein comprises amino acids 281 to 455 of SEQ ID NO: 2, or a conservative amino acid substitution variant thereof.

5. The composition of claim 1 wherein said protein comprises amino acids 20 to 455 of SEQ ID NO: 2, or a conservative amino acid substitution variant thereof.

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Search Results - Record(s) 1 through 10 of 16 returned.

☐ 1. Document ID: US 6677432 B1

L9: Entry 1 of 16 File: USPT Jan 13, 2004

US-PAT-NO: 6677432
DOCUMENT-IDENTIFIER: US 6677432 B1

TITLE: Mutations of the C-terminal portion of TGF-.beta. superfamily proteins

DATE-ISSUED: January 13, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Oppermann; Hermann	Medway	MA		
Tai; Mei-Sheng	Shrewsbury	MA		
McCartney; John	Holliston	MA		

US-CL-CURRENT: 530/350; 435/440, 435/445, 435/69.1, 530/399, 536/23.4

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

☐ 2. Document ID: US 6495513 B1

L9: Entry 2 of 16 File: USPT Dec 17, 2002

US-PAT-NO: 6495513
DOCUMENT-IDENTIFIER: US 6495513 B1

TITLE: Morphogen-enhanced survival and repair of neural cells

DATE-ISSUED: December 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rueger; David C.	Hopkinton	MA		
Kuberasampath; Thangavel	Medway	MA		
Oppermann; Hermann	Medway	MA		
Ozkaynak; Engin	Milford	MA		
Pang; Roy H. L.	Etna	NH		
Cohen; Charles M.	Medway	MA		

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US-CL-CURRENT: 514/2; 514/12

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 3. Document ID: US 6399569 B1

L9: Entry 3 of 16 File: USPT Jun 4, 2002

US-PAT-NO: 6399569
DOCUMENT-IDENTIFIER: US 6399569 B1

TITLE: Morphogen treatments for limiting proliferation of epithelial cells

DATE-ISSUED: June 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cohen; Charles M.	Medway	MA		
Charette; Marc F.	Needham	MA		
Kuberasampath; Thangavel	Medway	MA		
Rueger; David C.	Hopkinton	MA		
Oppermann; Hermann	Medway	MA		
Pang; Roy H. L.	Etna	NH		
Ozkaynak; Engin	Milford	MA		
Smart; John E.	Weston	MA		

US-CL-CURRENT: 514/12; 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 4. Document ID: US 6211146 B1

L9: Entry 4 of 16 File: USPT Apr 3, 2001

US-PAT-NO: 6211146
DOCUMENT-IDENTIFIER: US 6211146 B1

TITLE: 60A protein-induced morphogenesis

DATE-ISSUED: April 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kuberasampath; Thangavel	Medway	MA		
Pang; Roy H. L.	Etna	NH		
Oppermann; Hermann	Medway	MA		
Rueger; David C.	Hopkinton	MA		
Cohen; Charles M.	Medway	MA		

h e b b g e e e f e f ef b e

US-CL-CURRENT: 514/12; 424/422, 424/426, 424/484, 424/520, 514/2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 5. Document ID: US 6090776 A

L9: Entry 5 of 16 File: USPT Jul 18, 2000

US-PAT-NO: 6090776
DOCUMENT-IDENTIFIER: US 6090776 A

TITLE: Morphogen treatment of organ implants

DATE-ISSUED: July 18, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kuberasampath; Thangavel	Medway	MA		
Pang; Roy H. L.	Etna	NH		
Oppermann; Hermann	Medway	MA		
Rueger; David C.	Hopkinton	MA		
Cohen; Charles M.	Medway	MA		
Smart; John E.	Weston	MA		

US-CL-CURRENT: 514/2; 514/12, 514/8

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 6. Document ID: US 6077823 A

L9: Entry 6 of 16 File: USPT Jun 20, 2000

US-PAT-NO: 6077823
DOCUMENT-IDENTIFIER: US 6077823 A

TITLE: Method for reducing tissue damage associated with ischemia-reperfusion or hypoxia injury

DATE-ISSUED: June 20, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kuberasampath; Thangavel	Medway	MA		
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Oppermann; Hermann	Medway	MA		
Rueger; David C.	Hopkinton	MA		
Cohen; Charles M.	Medway	MA		
Smart; John E.	Weston	MA		

h e b b g e e e f e f ef b e

US-CL-CURRENT: 514/12; 514/2, 514/8

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw De

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☐ 7. Document ID: US 6022853 A

L9: Entry 7 of 16 File: USPT Feb 8, 2000

US-PAT-NO: 6022853
DOCUMENT-IDENTIFIER: US 6022853 A

TITLE: Morphogen-enriched dietary composition

DATE-ISSUED: February 8, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Cohen; Charles M.	Medway	MA		
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Oppermann; Hermann	Medway	MA		
Pang; Roy H. L.	Etna	NH		

US-CL-CURRENT: 514/12; 424/439, 424/464, 514/2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw De

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☐ 8. Document ID: US 5994131 A

L9: Entry 8 of 16 File: USPT Nov 30, 1999

US-PAT-NO: 5994131
DOCUMENT-IDENTIFIER: US 5994131 A

TITLE: Morphogenic protein screening method

DATE-ISSUED: November 30, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Oppermann; Hermann	Medway	MA		
Ozkaynak; Engin	Milford	MA		
Kuberasampath; Thangavel	Medway	MA		
Rueger; David C.	Hopkinton	MA		
Pang; Roy H. L.	Etna	NH		
Cohen; Charles M.	Medway	MA		

US-CL-CURRENT: 435/354; 435/325

h e b b g e e e f e f ef b e

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 9. Document ID: US 5972884 A

L9: Entry 9 of 16 File: USPT Oct 26, 1999

US-PAT-NO: 5972884
DOCUMENT-IDENTIFIER: US 5972884 A

TITLE: Morphogen treatment of gastrointestinal ulcers

DATE-ISSUED: October 26, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cohen; Charles M.	Medway	MA		
Charette; Marc F.	Needham	MA		
Kuberasampath; Thangavel	Medway	MA		
Rueger; David C.	Hopkinton	MA		
Oppermann; Hermann	Medway	MA		
Pang; Roy H. L.	Etna	NH		

US-CL-CURRENT: 514/12; 514/2, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 10. Document ID: US 5854071 A

L9: Entry 10 of 16 File: USPT Dec 29, 1998

US-PAT-NO: 5854071
DOCUMENT-IDENTIFIER: US 5854071 A

TITLE: OP-3- induced morphongenesis

DATE-ISSUED: December 29, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Oppermann; Hermann	Medway	MA		
Ozkaynak; Engin	Milford	MA		
Kuberasampath; Thangavel	Medway	MA		
Rueger; David C.	Hopkinton	MA		
Pang; Roy H. L.	Etna	NH		
Cohen; Charles M.	Medway	MA		

US-CL-CURRENT: 435/353; 435/325, 435/366, 530/350, 530/399

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Full | Title | Citation | Front | Review | Classification | Date | Reference | References | Attachments | Claims | KWIC | Draw De

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Search Results - Record(s) 11 through 16 of 16 returned.

☐ 11. Document ID: US 5741641 A

L9: Entry 11 of 16 File: USPT Apr 21, 1998

US-PAT-NO: 5741641
DOCUMENT-IDENTIFIER: US 5741641 A

TITLE: Morphogenic protein screening method

DATE-ISSUED: April 21, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Smart; John E.	Weston	MA		
Oppermann; Hermann	Medway	MA		
Ozkaynak; Engin	Milford	MA		
Kuberasampath; Thangavel	Medway	MA		
Rueger; David C.	Hopkinton	MA		
Pang; Roy H. L.	Etna	NH		
Cohen; Charles M.	Medway	MA		

US-CL-CURRENT: 435/6; 435/7.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | ... | Claims | KWIC | Draw De

☐ 12. Document ID: US 5739107 A

L9: Entry 12 of 16 File: USPT Apr 14, 1998

US-PAT-NO: 5739107
DOCUMENT-IDENTIFIER: US 5739107 A

TITLE: Morphogen treatment of gastrointestinal ulcers

DATE-ISSUED: April 14, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cohen; Charles M.	Medway	MA		
Charette; Marc F.	Needham	MA		

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Kuberasampath; Thangavel	Medway	MA
Rueger; David C.	Hopkinton	MA
Oppermann; Hermann	Medway	MA
Pang; Roy H. L.	Etna	NH
Ozkaynak; Engin	Milford	MA
Smart; John E.	Weston	MA

US-CL-CURRENT: 514/12; 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

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☐ 13. Document ID: US 5733878 A

L9: Entry 13 of 16 File: USPT Mar 31, 1998

US-PAT-NO: 5733878
DOCUMENT-IDENTIFIER: US 5733878 A
**** See image for Certificate of Correction ****

TITLE: Morphogen-induced periodontal tissue regeneration

DATE-ISSUED: March 31, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kuberasampath; Thangavel	Medway	MA		
Rueger; David C.	Hopkinton	MA		
Oppermann; Hermann	Medway	MA		
Cohen; Charles M.	Medway	MA		
Pang; Roy H. L.	Etna	NH		

US-CL-CURRENT: 514/12; 514/21, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

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☐ 14. Document ID: US 5707810 A

L9: Entry 14 of 16 File: USPT Jan 13, 1998

US-PAT-NO: 5707810
DOCUMENT-IDENTIFIER: US 5707810 A

TITLE: Method of diagnosing renal tissue damage or disease

DATE-ISSUED: January 13, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Smart; John E.	Weston	MA		

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Oppermann; Hermann	Medway	MA
Ozkaynak; Engin	Milford	MA
Kuberasampath; Thangavel	Medway	MA
Rueger; David C.	Hopkinton	MA
Pang; Roy H. L.	Etna	NH
Cohen; Charles M.	Medway	MA

US-CL-CURRENT: 435/6; 435/7.21

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC

☐ 15. Document ID: US 5656593 A

L9: Entry 15 of 16 File: USPT Aug 12, 1997

US-PAT-NO: 5656593
DOCUMENT-IDENTIFIER: US 5656593 A
**** See image for Certificate of Correction ****

TITLE: Morphogen induced periodontal tissue regeneration

DATE-ISSUED: August 12, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kuberasampath; Thangavel	Medway	MA		
Rueger; David C.	Hopkinton	MA		
Oppermann; Hermann	Medway	MA		
Cohen; Charles M.	Medway	MA		
Pang; Roy H. L.	Etna	NH		

US-CL-CURRENT: 514/12; 424/49, 514/21, 514/900, 514/902

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC

☐ 16. Document ID: US 5650276 A

L9: Entry 16 of 16 File: USPT Jul 22, 1997

US-PAT-NO: 5650276
DOCUMENT-IDENTIFIER: US 5650276 A
**** See image for Certificate of Correction ****

TITLE: Morphogenic protein screening method

DATE-ISSUED: July 22, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY

h e b b cg b cc e

Smart; John E.	Weston	MA
Oppermann; Hermann	Medway	MA
Ozkaynak; Engin	Milford	MA
Kuberasampath; Thangavel	Medway	MA
Rueger; David C.	Hopkinton	MA
Pang; Roy H.L.	Etna	NH
Cohen; Charles M.	Medway	MA

US-CL-CURRENT: 435/6; 435/29

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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